# COLD HARDINESS OF PASTURE GRASSES OF FLORIDA AS INFLUENCED BY NITROGEN FERTILIZATION

By

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# INTRODUCTION

With the increasing interest in the production of high-protein forage by means of heavy applications of nitrogen to Pangolagrass (*Digitaria decumbens* Stent.) and Pensacola Bahiagrass (*Paspalum notatum* Flugge) pastures, there has arisen a need for information concerning their comparative susceptibility to damage caused by low temperatures when they are grown at these high nitrogen levels.

These two grasses are widely used in pasture programs in Florida, and on their ability to remain productive rests a large part of the economy of the livestock industry.

Pangolagrass originated in the Pongola River Valley in South Africa. It was introduced into Florida by the United States Department of Agriculture in 1935. The grass was named, in 1942, after its native region, but the word was misspelled (49).

Pensacola Bahiagrass was first observed in Pensacola, Florida, about 1941. This grass was generally believed to have been brought into Florida from Central or South America. In 1942, seed from plants growing near the docks and railroads in Pensacola were sent to the experiment station in Gainesville. By 1945, seed were being marketed (35).

In view of the warm climates in the lands to which these grasses are native, one could expect that they might not be very cold hardy species. That this is generally true of Pangolagrass is an accepted fact. Pensacola Bahiagrass, however, is known to be rather resistant to damage from cold weather. Staten (82) has observed that Pensacola Bahiagrass was able to withstand mild winters in central Oklahoma during which minimum temperatures were around 0°F. He also noted that temperatures well below zero killed the grass. In Georgia, frost injury in March has been known to delay grazing of Pensacola Bahiagrass (46).

While there is a vast amount of literature concerning the hardiness of plants, the particular objectives of the present study have not been thoroughly investigated.

This experiment was undertaken with the idea of obtaining a quantitative measurement of the degree of cold hardiness exhibited by these two species at specified levels of nitrogen fertilization under the naturally occurring climatological conditions of a Central Florida winter.

In view of the relationship between temperature and cold hardiness, certain microclimatic investigations have been undertaken in order to obtain a better understanding of the real temperature conditions existing in the experimental area.

# REVIEW OF LITERATURE

## Introduction

With the tremendous interest in cold hardiness, it is only to be expected that a great body of literature concerning the subject is in existence. Various persons from time to time have published rather extensive reviews. Rosa (64), in 1921, presented a wide review of the literature up to that period. Twenty years later a classic review was published by Levitt (41) who later reworked it and republished in 1956 (42). Also, in 1956, Dexter (22) made an excellent summary of work dealing with winter hardiness and evaluation of crop plants as to winter hardiness.

There is some tendency to use the terms "winter hardiness," "cold hardiness," and "frost hardiness" interchangeably. Levitt (42) defined the winter hardiness of plants as their ability to survive the severities of winter. He assigned a numerical value to the frost hardiness of a plant. This value was the number of degrees (°C) below its freezing point at which a plant had to be frozen in order that a 50 per cent kill could be obtained.

Levitt (41) stated that Pfeiffer concluded that a chemical process must be involved in hardening, since the dehardening which occurs in the spring, when temperatures are high, is more rapid than in fall and winter.

Suneson and Peltier (85) found that the actual level of hardening of winter wheat was apparently determined by the efficiency and duration of the preceding growth-hardening stage.

Levitt (41) has observed that hardy plants have relatively small cells but that the converse is not necessarily true.

## Physiological Conditions Associated With Hardiness

Certain physiological conditions associated with cold hardiness have been listed by Meyer and Anderson (55). These include relatively low water content of the tissues, an accumulation of soluble carbohydrates in the cells accompanied by an increase in their osmotic pressure, and an increased proportion of unfreezable or bound water.

A number of experiments reviewed by Levitt (41) indicated that low environmental moisture is favorable to frost resistance while high soil moisture tends to prevent hardening.

Newton and Brown (57), studying seasonal changes in the composition of winter wheat in relation to frost resistance, observed that the reduction in moisture content is one of the most important changes in the quantitative relations of the various plant constituents. They noted that this occurs to a greater degree in hardy varieties.

It has been suggested by Dexter (19) that the development and maintenance of a high available carbohydrate supply along with a retarded vegetative growth, is necessary for an efficient cold-temperature hardening reaction.

Cold hardiness was observed to have no correlation with reducing sugars in work reported by Wood and Sprague (99) on ladino clover. However, in this study they found a high positive correlation of cold hardiness with total polysaccharides and hemicellulose contents but lower correlations with starch and total available carbohydrates.

Ensen and Blish (30) established a parallelism between increases in dry-matter and sucrose content and in cold resistance increase. They noted that with dehardening there was a progressive decrease in dry-matter and sugar content. This work was done on wheat.

With the conversion of starch to sugars, cold resistance was found to increase rapidly in certain legumes studied by Bula and Smith (8). They found the rate of metabolic activity to be inversely proportional to cold hardiness.

In work with black locust trees, Siminovitch and Briggs (78) have suggested that reversible changes in frost hardiness may be due to temperature-sensitive variations in starch content of the tissues. Ruelke and Smith (65) working with white, ladino, and red clovers observed that during the winter there was a decline in total available carbohydrates. Total sugars were found to be at a higher level during the period of winter dormancy in those species which were were hardy.

In a comparison of cold hardy and non-hardy red clover roots, Greathouse (29) found indications that hardiness was associated with greater concentration of carbohydrates and nitrogen, lower moisture content, larger unfreezable water value, lower specific conductance value, and a slightly higher pH value. He also noted that some difficulty arises in distinguishing cause from effect in cold hardiness studies.

Stark (80) defines the term "bound water" to mean water held against some extracting force. Grandfield (28), working with crown buds of alfalfa, noted that high nitrogen content of the crown bud might be partly responsible for their high content of bound water. He found that during the hardening period a decreased percentage of total water was associated with the increased concentration of sugars as well as the high nitrogen content of the buds. A further binding of the free water with protoplasmic compounds was the result.

Rosa (64) stated that the chief factor in increasing the osmotic concentration in plants is considered to be the decrease in the amount of free water, and that, therefore, the observed increase in osmotic concentration would be a secondary result of the hardening process.

Scarth and Levitt (70) reviewed cell changes in hardening and discussed various theories concerning the mechanism of frost resistance. They found that in hardened cells the resistance to injury by deplasmolysis is greater. In woody and herbaceous species osmotic pressure was reported as being increased in hardened cells. Colloids and bound water increased in hardened cells.

Various workers have attempted to find some correlation between cold hardiness and various nitrogen fractions present in plants including proteins. Hodgson and Bula (33) studied the hardening behavior of sweetclover varieties and discussed the possible role of various nitrogen fractions in cold resistance development. It appeared that water-soluble protein was most closely associated with cold resistance.

Newton and Brown (57) working with winter wheat found that amino nitrogen and non-coagulable nitrogen increase during the cold season, but found no correlation between the apparent degree of protein-splitting and the relative hardiness of varieties.

In ladino clover stolons, Wood and Sprague (89), working in New Jersey, observed that total nitrogen increased during the first half of the winter and then leveled off, but that there was no correlation with cold hardiness.

Siminovitch and Briggs (73, 75) studied seasonal changes in water-insoluble protein nitrogen, water-soluble protein nitrogen, and water-soluble non-protein nitrogen in relation to seasonal variations of frost hardiness in living bark tissue from black locust trees. They found a correlation only between the changes in water-soluble protein and hardiness. The correlation was positive and suggested to them that this constituent of the bark may bear some casual relationship to the mechanism of frost hardiness. In another work, Briggs and Siminovitch (8) studied seasonal changes in the electrophoresis patterns of water-soluble proteins from locust bark. They were able to distinguish at least five components. When hardiness and concentration of total protein were showing maximum variation in fall and spring they could find no real deviations in the electrophoresis patterns from those which characterize the winter protein. There were marked changes in summer.

Mark (47), studying the relation of reserves to resistance to cold in alfalfa, observed no consistent differences between hardy and non-hardy varieties as to soluble nitrogen. He found that soluble nitrogen increased in all varieties during late fall but that there seemed to be no evidence of protein hydrolysis since both protein and non-protein nitrogen were accumulating at the same time.

Newton and Brown (53) studied frost precipitation of proteins of plant juice and concluded that dehydration was the basic cause. When Newton *et al.* (55), exposed press-juice from unhardened winter wheat plants to frost they obtained a decrease in coagulable protein content and an increase in amino nitrogen content. There was also an increased sensitivity to hydrolysis by dilute sodium hydroxide. They suggested that protein splitting such as is found in hardened plants could be the result of frost rather than an adaptation against it.

In work with alfalfa, Dula _et al_. (7), found that there seemed to be some correlation between trends of water-soluble protein nitrogen and cold resistance in certain varieties but not in another which was suspected of possessing a rather low inherent ability to develop resistance to cold.

Dexter (20) has said that evidently there is a series of reactions which occur in plants in winter regardless of an increase in hardiness. Soluble organic nitrogen is increased. Respiration rate is decreased but is not correlated with changes in amount of sugars or with enzyme activity. Sugars usually are increased and may or may not be accompanied by increased hardiness.

The actual cause of hardiness is complex and many environmental factors may induce changes in hardiness. Weimar (94) withheld light from alfalfa plants for a period of seven days. This caused the complete disappearance of starch and greatly retarded their ability to withstand freezing. Suneson and Peltier (80) showed a reduction in hardiness of winter wheat plants under controlled conditions of reduced light.

Wheat seedlings which were shaded during half of each day for eight days were found to be more tender than seedlings receiving full sunlight, in work reported by Dexter (18). Hardening could not be accomplished in the dark. When illumination was made available only during warm periods, there was more tendency for plants to harden than when illumination was given only during cold periods.

Tysdal (87) obtained better survival from frost when he allowed plants to harden under white light than when hardened under blue or red light.

### The Process of Freezing in Plants

Asahina (2) has studied the process of freezing in plant cells in great detail. He has observed that the temperature at which a spontaneous freeze may occur in plant tissues depends on such variables as the cooling rate, volume, water content, and the conditions of the tissue surface.

A rather good discussion of the process of freezing of plant tissues has been given by Siminovitch and Briggs (74) who state that there are two mechanisms by which plant tissues may freeze and two modes of injury. The first mechanism is triggered by slowly dropping temperatures which cause formation of extracellular ice crystals.

A rapid drop in temperature will cause intracellular freezing. The latter is always fatal since there is a mechanical disorganization of the protoplasm caused by the ice crystals. However, extracellular freezing only acts to concentrate the cell contents by dehydration, and injury arises from the effect of dehydration on the protoplasm and protoplasmic membranes of the cells.

Levitt (42) states that the frost-hardy cell has two lines of defense. The primary defense against injury is the development of protoplasm which can resist the mechanical stresses during freezing. The second includes various means of reducing the stresses the cell is subjected to when frozen. Included among the latter are: reduced cell size, less rigid cell walls, and higher cell permeability.

## The Importance of Nitrogen Fertilization in Obtaining High Yields

In 1857, Lawes, Gilbert, and Pugh (14) of the Rothamsted Experiment Station definitely established that nitrogen is essential to plants. A large body of literature has evolved in which various references are made to nitrogen and its effect on plants. High yields and high protein content are generally dependent on adequate supplies of nitrogen being available to plants.

Chamblee (10) found that the nitrogen content of orchardgrass was increased the first season, four to six weeks following application of 100 pounds of nitrogen. There was a smaller magnitude of increase in the second and third seasons.

McCreery and Stelly (51) observed significant differences in yield of pasture herbage attributable to nitrogen fertilization. That heavy applications of nitrogen fertilizer produce large increases in growth of bromegrass on nitrogen deficient soils was shown by Russell et al. (66). They also found a higher nitrogen content in the plants, at all stages of growth, which had received fertilizer.

A number of recent experiments have been reported in which rather high rates of nitrogen have been utilized in grass production programs. Vicente-Chandler and his associates (88) have applied nitrogen up to rates of 2000 pounds per year to the tropical grasses, Napiergrass, Guineagrass, and Paragrass. They found that they could obtain increased yields up to rates of 800 pounds per acre. Crude protein contents increased with the increased fertilization up to the highest rate tested.

Pangolagrass, Napiergrass, and Guineagrass were fertilized with rates of nitrogen up to 1000 pounds per acre by Little et al. (44). In their study Pangolagrass was the lowest producer and responded strongly only to the 200 pound level of nitrogen.

Blue and Gammon (4) found that when they applied nitrogen to Pangolagrass at rates up to 268 pounds per acre they obtained a linear yield response. The forage was also observed to increase in nitrogen content. In still another year when low leaching from lack of intense rains prevailed and with some carry-over of nitrogen they got no yield response to nitrogen.

Yields of dry matter and crude protein content of Pangolagrass and Coastal Bermudagrass were found to be increased by increased rates of nitrogen in work reported by Racine (61). In both of these grasses he observed that the forage which was harvested in an advanced state of growth contained a lower percentage of crude protein than forage harvested before reaching an advanced state of growth.

Gammon and Blue (26) by applying nitrogen in four equal increments during the growing season were able to increase the nitrogen content of Pangolagrass from 1.4 per cent at 96 pounds of nitrogen per year to 2.1 per cent at 288 pounds of nitrogen per year.

Marchant (46) obtained satisfactory response from Pensacola Bahiagrass to nitrogen fertilization, within certain limits. In this work nitrogen was applied in the spring and again in the summer.

Pensacola Bahiagrass was observed by Jeffers (34) to be consistently superior to both Coastal Bermudagrass and to Argentine Bahia in response to high nitrogen levels.

In work with Pensacola Bahiagrass and Pangolagrass, Wallace et al. (92) found the nitrogen content of plant material to be higher under the higher rates of nitrogen fertilization. They found that there was some variation between years as to the optimum time for application, rate, and source of nitrogen fertilization.

Volk (89) observed a monthly variation in yield and nitrogen content of Pensacola Bahiagrass and Pangolagrass in 1/2000 acre lysimeters. He found that under a limited nitrogen supply, Pangolagrass will produce a forage with a lower nitrogen content than will Bahiagrass.

In comparison of the relative efficiency of Pangolagrass and Bahiagrass as to nitrogen use, Wallace et al. (92) reported that Bahiagrass was more efficient at lower levels and Pangolagrass more efficient at higher levels insofar as production of dry forage and protein were concerned. On an average they reported Pangolagrass as being the more productive of the two grasses.

Killinger (38) has commented that as late as the end of 1958 there was no certainty as to how much fertilizer, particularly nitrogen, could be applied profitably to such grasses as Pangolagrass and Pensacola Bahiagrass.

## Nitrogen Fertilization in Relation to Cold Hardiness

Numerous reports can be found in the literature concerning nitrogen and its effect on cold hardiness. Studies of this nature have been made with many types of plants.

Edgerton (23) applied nitrogen to apple trees in the fall and early winter for a number of years in order to evaluate its effect on cold hardiness. He made observations on nitrogen accumulation and on the hardiness of the twigs and bark. October and early November applications of nitrogen were thought to have increased the susceptibility of both the twigs and bark to freezing.

Hardening of winter wheat plants of a hardy variety could not be obtained in the dark at 2° C. when they were grown with excess nitrogen. This work was reported by Dexter (20) who also reported that he was able to obtain hardening of plants grown at low rates of nitrogen. Both groups of plants showed increases in soluble organic nitrogen during the period at the low temperature. Differences in hardening were more marked than differences in nitrogen content. Under conditions of light, hardening could be obtained with both groups of plants.

Blackman (3) working in England on the growth of pastures in the spring found that no growth took place below a soil temperature of 42° F. Between 42° and 47° F. he noted that "nitrogen-manured" pasture grew at a greater rate than his control while at higher temperatures the growth rates were rather similar. Nitrogen content was increased significantly four to eight days after application even at soil temperatures below 37°. At this temperature, no growth was occurring.

White (86) conducted an intricate experiment involving rate and frequency of application of fertilizer. He applied nitrogen at rates higher than 6000 pounds per acre per year to Bermudagrass varieties used in golf greens. He found that plots developed a greyish-green color following a frost but recovered in three days if warm weather followed. The two varieties studied were found to differ in their reaction. He noted that Everglades 1 was less affected by drought and cold weather and was more tolerant of high fertility rates than Bayshore.

Creel (15) found winter-killing of Pangolagrass to be directly related to the level of nitrogen fertility. At rates higher than 224 pounds per acre per year he observed that Pangolagrass was severely winter-killed in the Gainesville, Florida area. Plants which received nitrogen at rates of 447 or more pounds per acre annually had more of a tendency to resume growth during warm periods than those fertilized at lower rates. In another work McCloud and Creel (50) reported that winter-killing of Pangolagrass was apparently associated with insufficient root reserves and that death of entire plants was observed after alternating periods of plant growth followed by freezing. In still another report on this work McCloud (48) comments that winter-killing of Pangolagrass was 100 per cent at levels of 60 pounds of nitrogen per acre applied bi-weekly.

Various reasons have been advanced as to why nitrogen has an effect on hardiness. Levitt (41) reported that Hedlund stated that a rich supply of nitrogen increased growth and therefore decreased dry matter content and hardiness. Arland was said by Levitt (41) to have observed that nitrogen fertilization and potassium deficiency lead to decreased frost resistance due to a high transpiration rate. A higher water content is produced in plants by the former, but the latter has no effect on this factor. Kimball (37) recorded that the greatest tenderness was to be found among those herbaceous plants fertilized with sodium nitrate. Megee (54) tested naturally hardened alfalfa roots during fall and winter and found no difference in hardiness between plants fertilized with 0-8-24 in late May, those fertilized with nitrate of soda in late September, or the unfertilized controls.

Carroll and Welton (9) investigated the effect of heavy and late applications of nitrogen on Kentucky Blue Grass. In the unhardened condition no difference in frost resistance could be detected, but when both were hardened, the high nitrogen plants proved to be less resistant.

Kuksa was reported by Levitt (41) to have been able to produce a marked increase in the hardiness and total sugars of winter wheat by application of phosphorus and potassium, but when he included nitrogen, especially in large amounts, hardiness was decreased.

Levitt (41) stated that Lidforss explained the tenderness resulting from nitrogen fertilization as being due to a sugar deficiency and an increased protein concentration.

Although excess nitrogen generally reduces hardiness and deficiency increases it, Levitt (41) has observed that too great a deficiency may sometimes reduce hardiness.

## Temperature As a Factor in Cold Hardiness

Bula et al. (7) found temperature and day length to be major climatic factors which influenced the development of hardiness. They found that cold resistance in alfalfa developed more rapidly under conditions of shorter days and lower temperatures. Harvey (31) observed that loss of hardiness at greenhouse temperatures occurred at about the same rate as its gain at low temperatures. He found that he could obtain as much effect by an hour's exposure to cold as by a day's exposure, which led him to conclude that hardening is a cold shock response which is not related to the product of temperature and time of exposure. This work was done with tree seedlings. It was also noticed that alternating temperatures in certain cases induced a greater resistance than conditions of continuous exposure to low temperatures. Dexter (19) found, however, that alternating temperatures were no more effective in the hardening of alfalfa than continuous temperatures.

An increased hardening was observed by Tysdal (87) in alfalfa with each progressively lower temperature from 68° F. to 32° F. The maximum acceleration occurred at about 41° F. He obtained the highest degree of hardening when he alternated temperatures of slightly above 32° F. for sixteen hours with temperatures of 68° F. for eight hours and provided light during the warm period. His suggestion was that physico-chemical changes occur more rapidly at the high temperatures after stimulation by the low.

Peltier and Kiesselbach (61) and Suneson and Peltier (84) found that alternating temperatures were less effective for hardening of grains than constant low temperatures. This was assumed to be due to the continued growth under the conditions of alternating temperatures.

In November and early December, Suneson and Peltier (85) found that high daily temperatures in conjunction with high radiation were most conducive to hardening of winter wheat. These conditions were such as to give a maximum accumulation of organic reserves.

Salmon (69) stated that at greenhouse temperatures, winter hardened grain plants lose a perceptible amount of hardiness in a period of twenty-four hours, and most of their hardiness in 120 hours. Laude (40), however, showed that the process is not complete until twelve or fifteen days have passed.

Dexter (21) states that rehardening after vernalization is possible but more likely in unvernalized plants.

The killing point of peach fruit buds was found to fluctuate directly with changes in temperature during winter in work performed by Chaplin (13). He found that the killing point of the buds could rise as much as eleven degrees following a warm period and fall as much as five or six degrees following a period of low temperatures.

Meader and Blake (53) in other work with peach buds observed that more injury resulted when there was a quick drop to sub-zero temperatures following a period of thawing temperatures than when the coldest weather reached a minimum temperature through a process of gradual cooling over a period lasting several days. Meader (52) later made further reports emphasizing the importance of the rate of temperature fall in the development of cold injury to fruit buds of peaches. At the same rate of cooling he observed that a drop of one degree Fahrenheit within the critical temperature range brought about a significant increase in injury to buds of the varieties he tested.

Trends in cold hardiness of pine and fir were tested by Parker (60) who observed that there was a slow increase in hardiness during the later part of summer and the first part of autumn with a rather sharp increase in early November which coincided with nights in which temperatures were near or below freezing. In the middle of winter, leaves could withstand extremely cold temperatures without being damaged. With warmer days in February, there was a general decreasing of hardiness, but the degree of sensitivity exhibited in May was less than that shown by new needles appearing at that time of the year.

Wilson (98) has observed that under such conditions of low temperatures as occur in the arctic in the summer, there is a great sensitivity of the activity of plants to even minor temperature changes.

Meyer and Anderson (55) state that plants almost invariably will cease growth at temperatures somewhat above the minimum that can be withstood without injury. They also point out that temperature changes do not equally affect any two metabolic processes occurring in plants.

Rolfs (63) found that Rhodesgrass could survive temperatures as low as 17° F. provided an abundance of moisture was present and that the period of cold did not greatly exceed a week. If, however, frequent cold periods occurred accompanied by dry soil conditions, the grass could not survive temperatures far below 25° F.

Chandler (11) has shown that the resistance of plants to low temperature was not necessarily increased by rapid wilting of tissue. This was in comparison to dry unwilted tissue. He observed that tissue with a wet surface was more damaged by a given temperature than that which was devoid of surface moisture. Under conditions of slow wilting or long periods during which water was partially withheld, resistance of plants to low temperatures was increased. Plants tested included certain fruits, ornamentals, vegetables, and clover.

Some indication that freezing injury to oats may be dependent on the height of the stubble at the time of the freeze was noted by Neveland (33). He observed that recovery power of oats which had been not closely just prior to a freeze was less than that of oats cut closely just after a freeze. His readings of minimum daily temperatures indicated that when very cold conditions prevailed, the air between plants with relatively high stubble was two or three degrees warmer than where stubble was short.

Although air temperatures give some indication of what the situation is insofar as plants are concerned, it does not necessarily follow that leaf temperatures are the same as that of the surrounding air. It is possible to present conflicting evidence in regard to this variation in leaf and air temperatures, but generally the conflicts can be resolved by a strict interpretation of the conditions of measurement of the temperatures.

Geiger (27) stated that, in general, plants do not have the same temperature as the surrounding air. They are warmer than the air when the ground surface is warmer than the layer of air which is resting upon the ground.

This condition occurs during the day when there is a positive radiation balance. At night, when the radiation balance is negative, plants are usually cooler than the air.

Differences between plant and air temperatures disappear if the plant does not carry on its own radiation exchange. This is true during times of transition from positive to negative radiation balance and vice versa. This occurs at evening and morning, when the sky is completely overcast, and when there is rain, driving snow, or fog. These differences between air and plant temperatures also disappear if parts of a plant are screened by other parts.

Shaw (71) found that air temperatures in a standard instrument shelter were several degrees warmer than those of the leaves of certain plants when conditions were such as to favor strong upward radiation from the leaf. These differences were very much reduced when the sky was overcast. Some leaves exhibited undercooling with a later rise in their temperature which was interpreted to be due to the liberation of heat of fusion at the time of freezing.

Miller and Saunders (56) did some early work in which they studied temperatures of crop leaves. They found the temperatures of the upper surfaces of leaves of corn, sorghum, cowpeas, soybeans, watermelon, and pumpkin growing under field conditions in Kansas were approximately the same as those of the surrounding air. Under the same conditions, alfalfa leaves had a temperature slightly lower than that of the air.

Waggoner and Shaw (90), in a study of the relation of leaf orientation to temperature, found that a leaf which was perpendicular to insolation was about three degrees centigrade warmer than one oriented parallel to insolation. Their observation as to shading effects was that leaves which were shaded were of the same temperature as different parts of the plant, whereas those exposed to insolation were warmer. Exposure near the soil line was found to be conducive to higher temperatures than exposure near the top of the plant. Exposure was observed to be more important than plant type since no temperature differences were observed between similarly exposed potato and tomato leaves. In another work (91) these authors studied differences between plant temperatures and soil temperatures. They found that temperature differences between plant and environment disappeared at a depth of sixteen centimeters in the soil.

Wilson (98) found that in the arctic the maximum difference between leaf and air temperatures at midday is somewhat less than the differences which occur in temperate and tropical regions.

Kinbacher and Jensen (36) have advanced the idea that the microclimate at plant level is of the utmost importance in studies of winter hardiness. Along with others they belive that official temperature records are limited at times in their application to hardiness investigations.

Clarke (13) had arrived at the same conclusion somewhat earlier when he observed that minimum or damaging temperatures in orchards are not likely to be the same as those of the nearest weather station or even those at the buildings near the orchards, and that large deviations may occur within one orchard. He also noted that apparently minor differences in orchard sites may have considerable effect on the relative freedom or liability of the trees to frost damage.

Sprague (78, 79) and his associates have made a study of air temperature in the microclimate at various stations in the northeastern part of the United States. Their investigations involved a comparison of temperatures at the three-inch level with those at the five-foot level over a bluegrass sod. The daily range of temperature at the lower level was found to be considerably greater than at the higher level. Differences were less in the winter than in the summer.

### Methods for Determining Cold Hardiness

Various methods have been used to evaluate plants as to cold hardiness. Observations of a plant's ability to survive a winter in the field have probably been most widely used in testing for cold hardiness. Although this is good in that it tests plants under natural conditions, it has the disadvantage of the possibility of long periods of mild winters before a real test winter occurs.

Briggs and Siminovitch (8, 73) have used per cent of survival of cells after dehydration by plasmolysis as a cold hardiness test. In order to obtain plasmolysis, they subjected tissues to a graded series of strong salt solutions. They (74) have also used desiccation tests to determine hardiness. In these tests, cell survival was noted after desiccation in atmospheres of varied relative humidities. A graded series of sulphuric acid solutions were used to produce the different atmospheres.

Sakai (66) believed that Siminovitch's plasmolysis method involves some risk, since results obtained by Sakai indicated that the greater part of the difference of the dehydration resistance, obtained by the Siminovitch method, were due to the increase in the osmotic concentrations of the cells which resulted from the hardening treatment, artificial or natural.

Conductivity tests have been suggested by Dexter et al. (12, 17) as a means by which plants can be numerically rated as to cold hardiness. They have assumed that there is a correlation between the degree of injury from low temperatures with the exosmosis of electrolytes after plant cells have been exposed to cold. Electrical conductivity measurements are used to determine the amount of electrolytes which have diffused outward and, therefore, to estimate the degree of injury.

The conductivity method has been employed by numerous investigators in research involving various types of plants. Swingle (86) used the method in apple rootstock hardiness studies. He found that the time of exposure of test material to low temperatures was critical, but that the period for exosmosis was not too important. Stuart (82) employed this process to determine the reciprocal influence of stock and scion on hardiness of apple trees. Edgerton (23) also used it in studies of nitrogen's effect on cold hardiness of apple trees. In other work with woody plants, Wilner (96, 97) has advocated the use of the conductivity method as a test for hardiness. Sakai (67) used electrical measurements in a study of twig survival.

A number of workers, including Grandfield (28), Ruelke and Smith (66), Bula and Smith (6), Megee (54) and Silkett et al. (75), have used the conductivity method in their studies of cold hardiness of different forage plants.

Dexter (22) has taken Bula and Smith to task for an attempt to compare winter hardiness of species without determining percentage extraction of electrolytes. He indicated that in order to make comparisons it was necessary to allow for extraction of all electrolytes and determine the percentage which were extracted following the freezing treatment. Dexter used a process which involved the placing of the tubes containing the samples into boiling water in order to extract all the electrolytes. Boiling has also been used by Stuart (83) and by Emmert and Howlett (24).

A number of investigators have made conductivity readings of the tissue itself rather than of solutions. Among these have been: Dexter (18), working with alfalfa, Filinger and Seiger (25), working with apples, Campbell and Ghosheh (8), working with grapes, and Greenham and Daday (30), working with clover and alfalfa.

The work of Greenham and Daday is rather interesting in that it involves resistance readings utilizing high and low frequencies. This relates back to the early work of Luyet (45). He observed that resistance decreases at low frequencies with increased injury until the minimum resistance of dead tissue is reached, but that the resistance of dead and living tissue is nearly the same at high frequencies. Luyet worked with *Ambrosia trifida*.

Lewis and Tuttle (43) studied a number of plants from autumn until summer and observed very little variation in the concentration of electrolytes during the whole season.

## MATERIALS AND METHODS

### 1957-58

Grass samples were obtained from plots of Pensacola Bahiagrass (*Paspalum notatum* Fluegge) and Pangolagrass (*Digitaria decumbens* Stent) which were established in 1956. The plots were on Arredondo loamy fine sand on the Agronomy Farm, University of Florida, Gainesville. These plots have been constantly maintained at the specified levels of fertility since establishment. A spring application of 500 pounds of 6-6-6 fertilizer per acre was applied in late February. Equal applications of ammonium nitrate were applied in May, July, and September to Bahiagrass and in May, June, and July to Pangolagrass at a rate designed to provide yearly nitrogen levels of 100, and 400 pounds of nitrogen per acre per year. A photograph of the plots taken from the air is shown in Figure 1.

From the plots on which the tests were run, samples were obtained by removing a group of plants with a spade. These whole plants were transferred to the laboratory and thoroughly cleaned with tap water.

Test samples of Bahiagrass were prepared by removing all roots and all rhizomes, except for a one-inch portion of rhizome just below the crown. All top growth extending more than one and one-half inches above the crown was then removed.

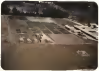

Fig. 1.--A view of the grass plots taken from the air showing the general layout. The weather station is in the lower right foreground.

The test samples were rinsed three times in deionized water, wrapped in cheesecloth and dried by a slinging motion.

The same procedure was followed for Pangolagrass samples except that roots were removed up to the crown and the above-ground growth left was approximately two and one-half inches in length.

Kneen and Blish (39), in justifying hardiness studies on crowns of wheat said the following:

> The cold resistance of neither the roots nor the leaf blades is a reliable index of the cold resistance of the plant. Survival seems to be entirely dependent on the ability of the crown to resist frost injury.

They did state, however, that the survival of the leaf contributes to the physiology of cold resistance.

Examples of the type of crowns used in this study are shown in Figures 2 and 3.

Fig. 2.--Pensacola Bahiagrass crowns used in the conductivity tests.

Fig. 3.--Pangolagrass crowns used in the conductivity tests.

The samples were weighed on a torsion balance, and five grams of material were placed in 25 x 200 millimeter test tubes and lightly corked. Nine tubes were prepared for each fertilization treatment in each grass. Three of these nine were kept at room temperature for 24 hours to serve as checks while the remaining six were submitted to a cold treatment of 25° F. for the 24 hour period. The cold treatment was administered in a deep freeze.

A 24 hour test period was selected as a result of previous work which indicated that this period and this temperature gave the most reliable results.

The tubes were placed in the freezer in wooden test tube holders. These test tube holders were placed on a freezer shelf beneath which an electric fan was mounted. The fan provided an even flow of air and maintained an even temperature. A Brown potentiometer, operating with a sixteen-point recording system of copper-constantan thermocouples, was used to check the uniformity of the air temperature.

At the termination of the 24 hour period, 50 milliliters of deionized water were delivered to each tube by pipette and a period of 20 hours was allowed for diffusion at room temperature.

After the diffusion period, all samples were brought to and maintained at 25° C. in order that resistance readings could be taken with a Model C [illegible] Bridge. Readings of resistance were taken on the solutions from the tubes. Resistance readings were also taken on a sample of the deionized water used at each testing period. This served as a check on the purity of the water. A determination of the cell constant was made at the time of each test by taking a resistance reading on a sample of 1/50 normal potassium chloride solution and multiplying this reading by the specific conductivity of the solution.

Specific conductance of the samples was computed by dividing the number obtained as the cell constant by the measured resistance of the sample.

After the readings were obtained, all plants from the check tubes of each fertility level in each grass were mixed together and a random sample of 10 plants was drawn from each of these groups. This sample was planted in the greenhouse. The same procedure was followed with the treated plants. A period of 21 days was allowed to elapse and, at the end of this period, the number of living plants was ascertained. At each fertility level, the per cent survival represented the percentage of living treated plants after 21 days growth in the greenhouse. The number of living check plants was expressed as 100 per cent.

In order that hardiness might be compared to temperature, daily readings of the maximum and minimum temperature were obtained from the weather station located on the Agronomy Farm. This station was located within a few hundred feet of the grass plots. The readings were taken with United States Weather Bureau mercury thermometers in standard shelters at the standard height of five feet.

Further temperature checks were made in a parallel study of the microclimate. This study was carried on in the grass plot area and encompassed most of the days when very severe cold conditions existed. Dates of observation were chosen arbitrarily in a few cases, but they were generally selected as a result of warnings from the weather bureau as to expected cold weather.

Vertical levels of observation included measurements at soil surface, three inches in air, 12 inches in air, and five feet in air.

The temperatures were measured with the Brown potentiometer previously described. This instrument was connected to thermocouples which were mounted on a pole at the heights at which temperatures were recorded. When air temperatures were measured, the thermocouples were shielded by aluminum shield plates. The inner surfaces of these plates were painted with a flat black paint so that the thermocouples were maintained at air temperature. Soil surface temperatures were recorded by placing the thermocouple in contact with the soil surface.

Calibration checks were made from time to time by placing all the thermocouples in water or kerosene at various temperatures and checking the recorded temperature against the reading of a United States Weather Bureau thermometer.

The chart speed of the recording instrument was set at five inches per hour. The temperature measured by each thermocouple was recorded once every eight minutes.

## 1958-59

The experiment for this year was designed so that a statistical analysis could be made of the data. This analysis was based on the methods of Snedecor (77). A split-split plot design was used in which the main plots were certain periods of the season, subplots were the two grasses studied, (*Paspalum notatum* Flugge and *Digitaria decumbens* Stent.), and sub-subplots were the two fertility levels which included 100 and 400 pounds of nitrogen per acre per year respectively.

Three replications were included in the experiment and samples were removed from each consecutively with a group of three dates of sampling constituting a period.

Samples upon which conductivity readings were determined were prepared in the same manner as previously described for the 1957-58 experiment, except that each test tube contained plant material sampled from a different area of the same treatment. Six samples were removed from each of the two grass species and fertility levels on each test date.

Certain modifications were made in the procedure in that after resistance readings, following the freezing treatment, were taken on the solutions, the contents of two tubes were composited and placed in a Waring Blender in which the material was ground until a homogeneous mixture was obtained. This mixture was then filtered through a number one Whatman filter and a resistance reading was taken on the filtrate.

An average of the readings for the three sets of two-tube composite samples was obtained and from this the specific conductivity was calculated. This figure was taken to be the total conductivity for the grass from that particular fertility level on that particular test date. Utilizing this number and the specific conductivity determined for the individual samples following the freezing treatment, the per cent of the total conductivity was determined for the various samples. An average of these values for per cent of total conductivity was obtained and used as the value for the particular fertility level on the particular date in the statistical analysis.

Per cent of total conductivity was determined in order that the two grasses might be compared and that the two fertility levels might also be compared.

Determinations of total nitrogen content were made on part of the material obtained for each sample. These determinations were made for the last five periods.

Nitrogen analysis was by the Kjeldahl method (1).

Nitrogen content was also determined on samples of forage from each clipping date during the summer of 1958.

Yield data were taken at each clipping date during the summer of 1958 and at the first clipping date for 1959.

Temperature measurements in the microclimate were taken in the same manner as for 1957-58 except that four replications were used. Poles were set up at each corner of a four feet square as shown in Figure 4.

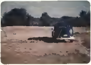

Fig. 4.--Temperature investigations in the microclimate.

## RESULTS

## 1957-58

### Climatic conditions during the 1957-58 season

The climatic data for the 1957-58 season are summarized in Table 1. They were obtained from the weather station located on the Agronomy Farm two and one-half miles from Gainesville Florida in Township 10 (south) Range 19 (east) Section 12. The exposure is over light sandy soil on moderate low ground in gently rolling country. Experimental plots were located approximately 100 yards north of this station at a slightly lower elevation.

TABLE 1

CLIMATOLOGICAL DATA 1957-58
AGRONOMY FARM, GAINESVILLE, FLORIDA

| Date | Average Temperature Max. (° F.) | Min. (° F.) | Mean (° F.) | Precipitation Total (Inches) | Total Wind Movement (Miles) |
|---|---|---|---|---|---|
| Oct. 1 - 7 | 81.1 | 64.1 | 72.6 | 1.61 | 170 |
| 8 - 14 | 80.7 | 58.7 | 69.7 | 0.01 | 330 |
| 15 - 21 | 85.6 | 56.7 | 71.0 | 0.55 | 320 |
| 22 - 28 | 73.6 | 53.6 | 63.6 | 0.12 | 360 |
| 29 -Nov.4 | 77.4 | 47.1 | 62.3 | 0.00 | 155 |
| Nov. 5 - 11 | 76.3 | 51.3 | 63.8 | 0.12 | 365 |
| 12 - 18 | 83.0 | 61.4 | 72.2 | 0.74 | 255 |
| 19 - 25 | 75.7 | 57.6 | 66.7 | 0.73 | 395 |
| 26 -Dec.2 | 71.0 | 46.3 | 58.1 | 0.53 | 335 |
| Dec. 3 - 9 | 65.7 | 40.6 | 53.0 | 0.76 | 300 |
| 10 - 16 | 61.6 | 30.0 | 45.8 | 0.07 | 445 |
| 17 - 23 | 73.6 | 40.3 | 56.0 | 0.55 | 340 |
| 24 - 30 | 73.6 | 36.0 | 55.0 | 0.04 | 265 |

The 1957-58 season was probably one of the most disastrous to Florida's agriculture in the twentieth century. Pasture grasses were subjected to repeated freezes, excessive rain, and high winds. These brought about severe losses. December, January, and February are alleged to have been the coldest consecutive three months which have ever been experienced in Florida. The severity of the winter caused the hardiness experiment to be abandoned in December because of the extensive damage done to the top growth of the grass.

## Bahiagrass

The data for Bahiagrass are summarized in Table 6. These data indicate that during the first three observation dates greater hardiness existed in the plants fertilized at the 400 pound level of nitrogen. In considering this, one might take into account the fact that no severe temperatures were experienced in the periods preceding these observations except during a short time before the observation made on December 6. The graph in Figure 3 will bring this picture more clearly into focus and will help to explain what occurred on the date of the last observation of the experiment. This last observation, made on December 16, indicates that ahigher degree of cold hardiness was then possessed by the grass grown at the 100 pound level of nitrogen. The fact that this later observation shows a reversal of the

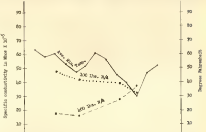

Figure 5.--Comparison of specific conductivity at the two fertility levels in Bahiagrass in 1957 with average weekly minimum temperature.

situation which prevailed in the three earlier observations might well be due to the period of low temperatures which were experienced just prior to the last observation date.

TABLE 6

AVERAGE SPECIFIC CONDUCTIVITY VALUES FOR PENSACOLA BAHIAGRASS AND PANGOLAGRASS AT LEVELS OF 100 AND 400 POUNDS OF NITROGEN PER ACRE DURING THE 1957-58 SEASON

| Field Sampling Date | Specific Conductivity (Mhos x $10^{-5}$) | | | |
|---|---|---|---|---|
| | Pangolagrass | | Pensacola Bahiagrass | |
| | 100 | 400 | 100 | 400 |
| October 31 | 79.88 | 73.76 | 50.04 | 45.67 |
| November 6 | 48.16 | 67.88 | 47.66 | 36.90 |
| December 6 | 90.11 | 61.91 | 40.22 | 50.07 |
| December 16 | 16.81 | 13.96 | 34.20 | 98.70 |

It is noted that while there is a continued decrease in the conductivity of the samples from the 100 pound level which would indicate a trend toward increasing cold hardiness throughout the winter, the opposite seems to be true for the grass grown at the 400 pound level. In the later case the trend seems to be that of a decreasing degree of cold hardiness throughout the season.

Very severe cold weather in December damaged the top growth of all of the grass to such an extent that the experiment was suspended on December 16.

Survival percentages are given in Table 3. These values are for the plants which were placed in the greenhouse after being subjected to the cold treatment. If the values for survival percentage are compared with those for specific conductivity, one may see that very good agreement is obtained insofar as their individual indication of the degree of cold hardiness being exhibited by the plants at a particular time. These figures place an increased emphasis on the fact that at the 400 pound level of nitrogen, Bahiagrass appeared to be decreasing in cold hardiness as the winter progressed.

TABLE 3

RECOVERY DATA FOR PLANTS PLACED IN THE GREENHOUSE AFTER THE FREEZING TREATMENT DURING THE 1957 SEASON

| Date | Grass | Fertility Level (lbs. N/A) | Survival Percentage | Specific Conductivity (Mhos x $10^{-6}$) |
|---|---|---|---|---|
| Oct. 21 | Bahiagrass | 100 | 0 | 48.16 |
| | Bahiagrass | 400 | 30 | 10.37 |
| | Pangolagrass | 100 | 0 | 40.10 |
| | Pangolagrass | 400 | 0 | 73.26 |
| Nov. 6 | Bahiagrass | 100 | 0 | 42.77 |
| | Bahiagrass | 400 | 20 | 10.25 |
| | Pangolagrass | 100 | 0 | 47.70 |
| | Pangolagrass | 400 | 0 | 66.70 |
| Dec. 4 | Bahiagrass | 100 | 0 | 40.11 |
| | Bahiagrass | 400 | 10 | 28.00 |
| | Pangolagrass | 100 | 0 | 50.00 |
| | Pangolagrass | 400 | 0 | 81.40 |
| Dec. 10 | Bahiagrass | 100 | 0 | 33.46 |
| | Bahiagrass | 400 | 0 | 38.64 |
| | Pangolagrass | 100 | 33 | 15.23 |
| | Pangolagrass | 400 | 40 | 13.03 |

Forage yields and winter injury data are given in Table 4. These data indicate that by the middle of April

TABLE 4

FORAGE YIELDS AND ESTIMATIONS OF WINTER INJURY

| Grass | Fertility Level (lbs.N) | Total Yield 1957 (lbs.) | Est. Winter Injury 4/14/58 (%) | Dry Matter Per Acre First Cutting 5/26/58 (lbs.) | Total Yield 1958 (lbs.) | Average Nitrogen Content (%) | Est. Winter Injury / /59 (%) | Dry Matter Per Acre First Cutting 5/26/59 |
|---|---|---|---|---|---|---|---|---|
| Pangolagrass | 100 | 5595 | 26.7 | 271 | 3128 | 1.43 | 6 | 497 |
|  | 400 | 11441 | 26.0 | 66 | 8192 | 2.47 | 13 | 737 |
| Pensacola Bahiagrass | 100 | 5975 | 0.5 | 1515 | 6345 | 1.26 | 2 | 867 |
|  | 400 | 13835 | 0.5 | 3968 | 15145 | 1.75 | 2 | 2467 |

on winter injury was evident in Bahiagrass. Total yields for the previous year are given in order that some comparison may be made between them and the data given for the first cutting following the winter. It is believed that data for this first cutting would give more indication of winter damage than from any other cutting. Yields are higher at the 400 pound level as would be expected. During the summer of 1957, the total yield at the 400 pound level of nitrogen was 2.2 times that at the 100 pound level. On the first cutting date in 1958, the yield at the 400 pound level was 2.6 times that at the 100 pound level. This would tend to indicate that there was no difference between fertility levels in the ability of the grass to survive the winter.

Pangolagrass

The results for Pangolagrass are summarized in Table 2 and Figure 6. Conductivity values were higher during the first three dates for samples of grass from the 400 pound level of nitrogen and lower for those samples from the 100 pound level. This would indicate, of course, that cold hardiness at this time was greater in the grass at the lower fertility level. On the last observation date, December 16, the reverse is true, but the difference in the conductivity values for the samples at the two fertility levels is extremely small. Insofar as the relative cold hardiness of

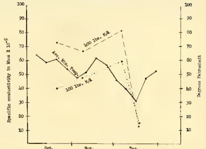

Figure 6.--Comparison of specific conductivity at the two fertility levels in Pangolagrass in 1957 with average weekly minimum temperature.

the two fertility levels is concerned, one can see that conditions which exist in Pangolagrass are just the reverse of what was found to be true of Bahiagrass.

Except for the slight increase in resistance, characterized by the fall in conductivity of the samples from the 400 pound level on the second observation date, November 8, there appears to be a condition in which there is continued decrease in cold hardiness throughout the season at both fertility levels until the middle of December. The last observation date, however, shows that an increase in cold hardiness at both levels occurred. This situation does not parallel that observed in Bahiagrass at all. The one point on which there seems to be a likeness is that for both grasses the conductivities of the samples from the two fertility levels do approach the same value on the last observation date when a period of extremely low temperatures had just previously occurred.

The cold hardiness studies were suspended in December because of severe weather which destroyed the top growth of the grass.

Data showing the amount of survival of plants placed in the greenhouse are given in Table 3. They indicate that the grass was lacking in cold hardiness until about the middle of December. This shows rather good agreement with the information obtained from measurements of specific conductivity.

Table 4 contains yield data and estimations of winter injury. Winter injury as determined in April was rather severe at both levels of fertility, but it was about three times more extensive at the higher rate of nitrogen. Winter survival comparisons of Pangolagrass are given in Figure 7.

Fig. 7.--Comparison of winter survival of Pangolagrass at the two rates of nitrogen fertilization. Severe winter-killing is indicated at the higher rate of nitrogen.

In the summer of 1957, the yield of Pangolagrass at the 400 pound level of nitrogen was 1.9 times that at the 100 pound level. On the first cutting date in 1958, the yield of Pangolagrass at the 100 pound level was 4.3 times the yield of Pangolagrass at the 400 pound level. This is the result which could be directly attributed to winter injury since the estimated winter injury at the 100 pound level of nitrogen was 26.7 per cent as compared to 80.0 per cent at the 400 pound level.

## Summary of 1957-58 experiment

Studies of cold hardiness in Pensacola Bahiagrass and Pangolagrass at fertility rates of 100 and 400 pounds of nitrogen per acre per year were conducted. These studies utilized the electrical conductivity method advanced by Dexter (17). Greenhouse studies of survival after freezing treatment were used to check the validity of the conductivity tests. Climatic data from the weather station located on the Agronomy Farm were measured and presented.

Measurements of specific conductivity indicated that during October and November and the early part of December the greater degree of cold hardiness was exhibited at the 400 pound level in Bahiagrass and at the 100 pound level in Pangolagrass. Following a period of low temperatures in early December, the degree of cold hardiness under the two fertility levels appeared to approach equal values in each grass but not between the two grasses.

Since the experiment was terminated in mid-December only trends can be observed. It appears, however, that when the testing ceased, Bahiagrass was increasing in cold hardiness at the 100 pound level of nitrogen and decreasing at the 400 pound level. Pangolagrass appeared to be increasing in cold hardiness at both fertility levels. Yield data taken in the spring of 1958 showed that winter injury was approximately equal at the two fertility levels in Bahiagrass but that it was considerably more severe at the

400 pound level in Pangolagrass. This later statement is in agreement with the findings of Crael (18) who observed that Pangolagrass was increasingly injured by cold with increased rates of nitrogen.

Good agreement was obtained between the electrical conductivity tests and the survival studies carried out in the greenhouse.

## 1958-59

### Climatic conditions during the 1958-59 season

The climatic data for the 1958-59 season are summarized in Table 5. They were obtained from the weather station located on the Agronomy Farm.

The winter was rather mild but somewhat damp. Temperatures dropped into the low thirties only about half as often as in the preceding winter. The first freezing temperatures did not occur until the last day of November. Photographs of the first frost and its effects are given in Figures 8 and 9. The middle of December was rather cold with several frosts. January was relatively cold and the season's lows were recorded in mid-January. February was slightly warmer than normal. March had a few cold nights.

Precipitation was about normal until February when it began to reach proportions far above normal.

### Bahiagrass as compared to 1957

The experimental emphasis for 1959 was placed on the results as indicated by per cent of total conductivity. However, in order that some comparison might be made between the results of the two seasons, a short resume is given concerning the conductivity values obtained during the part of the season which corresponds to that of the 1957-58 season. These data are summarized in Table 6 and Figure 10.

TABLE 6

CLIMATOLOGICAL DATA 1958-59
AGRONOMY FARM, GAINESVILLE, FLORIDA

| Date | Average Temperatures Maximum °F. | Minimum °F. | Mean °F. | Total Precipitation (Inches) | Total Wind Movement (Miles) |
|---|---|---|---|---|---|
| Sept. 1 - 7 | 92.0 | 71.4 | 81.7 | 0.22 | 306 |
| 8 - 14 | 91.4 | 69.3 | 79.9 | 0.85 | 200 |
| 15 - 21 | 93.0 | 71.4 | 82.4 | 0.10 | 160 |
| 22 - 28 | 88.0 | 70.1 | 80.1 | 0.39 | 345 |
| 29 - Oct. 5 | 88.4 | 65.6 | 77.0 | 0.44 | 265 |
| Oct. 6 - 12 | 86.1 | 60.0 | 73.0 | 0.04 | 410 |
| 13 - 19 | 80.0 | 63.1 | 71.0 | 0.00 | 465 |
| 20 - 26 | 79.6 | 52.7 | 66.1 | 0.00 | 670 |
| 27 - Nov. 2 | 78.0 | 38.4 | 58.0 | 1.41 | 225 |
| Nov. 3 - 9 | 74.8 | 56.1 | 65.4 | 0.30 | 170 |
| 10 - 16 | 81.6 | 69.0 | 75.3 | 0.00 | 260 |
| 17 - 23 | 81.0 | 53.0 | 67.0 | 0.00 | 190 |
| 24 - 30 | 75.0 | 59.0 | 67.0 | 0.06 | 300 |
| Dec. 1 - 7 | 73.7 | 52.7 | 63.0 | 1.28 | 270 |
| 8 - 14 | 63.4 | 43.0 | 53.0 | 0.67 | 200 |
| 15 - 21 | 66.6 | 34.3 | 50.3 | 0.54 | 310 |
| 22 - 28 | 66.3 | 47.0 | 56.1 | 0.58 | 345 |
| 29 - Jan. 4 | 65.8 | 50.3 | 58.1 | 0.67 | 300 |
| Jan. 5 - 11 | 62.0 | 35.0 | 48.5 | 0.06 | 390 |
| 12 - 18 | 63.6 | 30.4 | 47.0 | 0.68 | 310 |
| 19 - 25 | 71.7 | 58.1 | 66.0 | 0.33 | 470 |
| 26 - Feb. 1 | 79.3 | 53.1 | 66.7 | 0.88 | 290 |
| Feb. 2 - 8 | 78.0 | 54.1 | 66.0 | 0.01 | 500 |
| 9 - 15 | 83.0 | 61.8 | 72.4 | 0.04 | 345 |
| 16 - 22 | 73.0 | 58.0 | 61.0 | 0.00 | 475 |
| 23 - Mar. 1 | 71.4 | 53.0 | 62.0 | 0.34 | 390 |
| Mar. 2 - 8 | 71.3 | 45.0 | 58.1 | 1.02 | 630 |
| 9 - 15 | 73.7 | 44.4 | 59.1 | 3.54 | 390 |
| 16 - 22 | 68.4 | 51.0 | 60.0 | 0.05 | 465 |
| 23 - 29 | 78.1 | 38.0 | 58.0 | 0.15 | 440 |

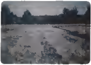

Fig. 8.--First killing frost in 1958-59 season.

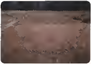

Fig. 9.--Grass plots three days after frost pictured above.

Similarities during the two seasons may be seen by comparing Figures 8 and 10. It is to be noted that, during both seasons, the grass grown under the 400 pound level of

## TABLE 6

### TOTAL NITROGEN CONTENT OF BAHIAGRASS AND CONDUCTIVITY OF PLANT TISSUE EXTRACTS IN RELATION TO NITROGEN FERTILITY LEVEL, 1958-59 SEASON

| Period | Date of Sampling in Field | Specific Conductivity of Tissue Extract (Mhos x $10^{-6}$) |  |  |  | Per Cent of Total Spec. Conduc. |  | Nitrogen Content (Per Cent) |  |
|---|---|---|---|---|---|---|---|---|---|
|  |  | After Cold Treatment |  | After Total Breakdown of Plant Tissue |  |  |  |  |  |
|  |  | 100 # N | 400 # N | 100 # N | 400 # N | 100 # N | 400 # N | 100 # N | 400 # N |
| 1 | Sept. 17 | 81.08 | 73.58 | 194.0 | 259.7 | 46.0 | 28.3 | - | - |
|  | Sept. 23 | 70.33 | 75.74 | 155.1 | 206.1 | 45.4 | 36.7 | - | - |
|  | Sept. 25 | 75.86 | 60.79 | 186.1 | 238.1 | 44.0 | 25.6 | - | - |
| 2 | Oct. 14 | 63.47 | 75.38 | 160.0 | 257.4 | 39.1 | 29.3 | 0.96 | 1.53 |
|  | Oct. 23 | 63.31 | 31.83 | 157.9 | 287.0 | 40.1 | 11.1 | 0.49 | 0.86 |
|  | Nov. 4 | 82.66 | 87.70 | 155.0 | 269.7 | 53.0 | 32.0 | 0.41 | 0.96 |
| 3 | Nov. 10 | 72.63 | 66.01 | 173.2 | 278.3 | 41.9 | 24.0 | 0.96 | 1.61 |
|  | Nov. 25 | 57.37 | 52.30 | 147.0 | 160.2 | 38.9 | 32.7 | 0.31 | 1.71 |
|  | Dec. 8 | 74.68 | 85.11 | 174.0 | 314.3 | 42.7 | 27.0 | 0.38 | 1.45 |
| 4 | Dec. 9 | 75.87 | 58.73 | 197.0 | 190.0 | 39.0 | 31.0 | 0.72 | 1.45 |
|  | Dec. 18 | 62.25 | 55.82 | 145.3 | 136.1 | 43.0 | 41.0 | 1.71 | 1.41 |
|  | Jan. 6 | 63.45 | 52.17 | 179.7 | 217.0 | 35.4 | 24.0 | 0.64 | 1.72 |
| 5 | Feb. 10 | 61.89 | 32.08 | 194.6 | 317.0 | 31.8 | 10.1 | 1.75 | 0.29 |
|  | Feb. 17 | 32.35 | 47.02 | 182.4 | 317.5 | 17.7 | 14.8 | 1.81 | 1.96 |
|  | Feb. 24 | 46.13 | 44.74 | 199.3 | 329.7 | 23.1 | 13.6 | 0.96 | 1.39 |
| 6 | Mar. 3 | 30.77 | 34.76 | 231.7 | 329.7 | 13.3 | 10.6 | 0.80 | 2.37 |
|  | Mar. 10 | 111.32 | 120.73 | 214.0 | 317.5 | 52.0 | 38.0 | 0.64 | 0.16 |
|  | Mar. 18 | 79.03 | 97.46 | 182.4 | 342.0 | 43.3 | 68.4 | 0.54 | 0.23 |

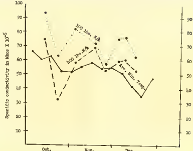

Figure 10.--Comparison of specific conductivity at the two fertility levels in Bahiagrass in 1958-59 with average weekly temperature.

nitrogen appears to possess the greater degree of cold hardiness. It is interesting to note that conductivities are considerably higher in general for the 1958-59 season than for the 1957-58 season. This higher conductivity would indicate a lesser degree of hardiness for the grass at both fertility levels during 1958-59. The difference in cold hardiness could be due to the difference in the weather conditions of the two years. Another difference between the two seasons is that in 1958 there is greater variation between readings for the various observation dates. It also appears that in 1958 the two fertility levels present the same tendency to vary in hardiness while in 1957 they are directly opposed in their tendency to vary. The small number of observations in 1957 may account for this later discrepancy.

### Pangolagrass as Compared to 1957

As in the case of Bahiagrass, the conductivity readings on Pangolagrass for the period corresponding to that of the previous season will be discussed. These data are summarized in Table 7 and Figure 11.

When the graphs in Figures 6 and 11 are compared, one observes that during both seasons greater cold hardiness is evident in the grass grown at the 100 pound level of nitrogen. The greater conductivity values observed in the 1958 season for Pangolagrass at both fertility levels

## TABLE 7

### TOTAL NITROGEN CONTENT OF PANGOLAGRASS AND CONDUCTIVITY OF PLANT TISSUE EXTRACTS IN RELATION TO NITROGEN FERTILITY LEVEL, 1958-59 SEASON

| Period | Date of Sampling in Field | Specific Conductivity of Tissue Extract (Mhos x 10⁻⁵) After Cold Treatment 100 # N | After Cold Treatment 400 # N | After Total Breakdown of Plant Tissue 100 # N | After Total Breakdown of Plant Tissue 400 # N | Per Cent of Total [illegible] 100 # N | Per Cent of Total [illegible] 400 # N | Nitrogen Content (Per Cent) 100 # N | Nitrogen Content (Per Cent) 400 # N |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Sept. 17 | 100.00 | 95.39 | 134.6 | 176.6 | 74.3 | 65.8 | - | - |
| | Sept. 23 | 78.40 | 82.68 | 133.8 | 147.8 | 60.8 | 68.8 | - | - |
| | Sept. 30 | 78.58 | 71.20 | 122.8 | 240.1 | 68.8 | 29.8 | - | - |
| 2 | Oct. 14 | 83.87 | 80.03 | 138.0 | 183.6 | 60.4 | 81.6 | 0.68 | 1.09 |
| | Oct. 23 | 60.69 | 80.90 | 128.3 | 174.8 | 47.1 | 39.4 | 0.78 | 1.28 |
| | Nov. 4 | 66.57 | 77.01 | 117.4 | 122.6 | 68.4 | 62.8 | 0.54 | 0.93 |
| 3 | Nov. 18 | 80.33 | 101.30 | 124.2 | 164.8 | 64.7 | 61.4 | 0.47 | 1.00 |
| | Nov. 25 | 68.86 | 68.11 | 126.1 | 120.7 | 34.8 | 49.8 | 0.51 | 1.26 |
| | Dec. 2 | 72.64 | 84.03 | 127.9 | 136.1 | 66.8 | 61.7 | 0.57 | 1.63 |
| 4 | Dec. 9 | 63.00 | 112.90 | 161.7 | 171.4 | 57.2 | 65.8 | 0.87 | 0.90 |
| | Dec. 16 | 68.00 | 78.37 | 127.8 | 124.2 | 54.8 | 63.8 | 0.70 | 1.53 |
| | Jan. 6 | 52.87 | 88.75 | 115.1 | 178.9 | 68.4 | 50.8 | 0.60 | 1.67 |
| 5 | Feb. 10 | 168.56 | 163.21 | 168.4 | 225.8 | 75.8 | 71.0 | 0.80 | 1.00 |
| | Feb. 17 | 117.68 | 122.45 | 164.8 | 190.8 | 71.2 | 64.3 | 0.68 | 1.30 |
| | Feb. 24 | 137.80 | 104.53 | 199.6 | 100.3 | 64.3 | 52.4 | 1.14 | 1.30 |
| 6 | Mar. 3 | 157.28 | 82.03 | 225.0 | 214.6 | 69.7 | 38.3 | 0.89 | 3.13 |
| | Mar. 10 | 139.37 | 176.73 | 238.1 | 285.7 | 60.5 | 61.9 | 0.63 | 3.68 |
| | Mar. 18 | 140.62 | 141.08 | 618.8 | 180.3 | 63.9 | 78.6 | 3.65 | 1.91 |

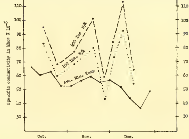

Figure 11.--Comparison of specific conductivity at the two fertility levels in Pangolagrass in 1958-59 with average weekly minimum temperature.

indicate that hardiness was greater during the 1957 season. The difference in hardiness is probably due to the difference in weather conditions in the two years. It may be well to note that this situation held true for Bahiagrass as well. In general, it can be said that there is not any large difference in the trends indicated by the two graphs.

## Hardiness as determined by per cent of total conductivity

As previously stated, the hardiness study during the fall and winter of 1958-59 was based on a determination of per cent of total conductivity. The study was divided into six periods with each consisting of three observation dates. These data are summarized in Tables 6 and 7 and Figure 12.

The data given in Table 6 indicate that there was a difference in the hardiness of the two grasses studied and in the hardiness exhibited at the two fertility levels. The interactions show that these differences varied with the periods and with the grasses.

In Figure 12, values of per cent of total conductivity are presented for the various periods. One may see from this graph that during the greater part of the season the highest values are exhibited by Pangolagrass. Higher values for per cent of total conductivity indicate less cold hardiness in the plants.

Since there were interactions between grasses and

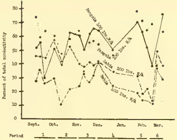

Figure 12.--Comparison of values of percent of total conductivity between the two fertility levels in the two grasses during the 1958-59 season.

periods and between fertility levels and periods, the various periods have been analyzed separately utilizing grasses as main plots and fertility levels as subplots. Significant interactions were found to exist between periods and grasses and between periods and fertility levels. No difference in the cold hardiness of either grass at either fertility level could be detected by the analysis insomuch as periodic differences in each individual entity are concerned.

TABLE 8

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF TWO GRASSES IN EACH OF SIX PERIODS AS AFFECTED BY FERTILITY TREATMENT DURING 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Replications | 2 | 73.89 | |
| Periods | 5 | 96.89 | |
| Error (a) | 10 | 3,558.24 | |
| Subplots: | | | |
| Grass | 1 | 11,265.70 | 11,265.70** |
| G x P | 5 | 1,991.78 | 398.36** |
| Error (b) | 12 | 993.82 | 82.74 |
| Sub-subplots: | | | |
| Fertility levels | 1 | 1,423.10 | 1,423.10** |
| F x P | 5 | 780.00 | 156.00* |
| F x G | 1 | 411.38 | 411.38** |
| F x P x G | 5 | 509.10 | 101.82 |
| Error (c) | 24 | 1,099.56 | 45.82 |
| Total | 71 | 21,808.78 | |

*Significant at 5 per cent level.

**Significant at 1 per cent level.

The analysis for the first period is given in Table 9. From these data and from the graph in Figure 12 it is possible to determine that in September greatest hardiness was exhibited by Bahiagrass at the 400 pound level of nitrogen. A lower degree of hardiness is exhibited by Bahiagrass at the 100 pound level. During this period, Pangolagrass was also more hardy at the 400 pound level of nitrogen. On the third observation date of the first period, the hardiness of Pangolagrass at the 400 pound level appeared to be greater than that of Bahiagrass at the 100 pound level.

During the second period, the analysis of which is given in Table 10, there was a highly significant interaction between grasses and fertility levels. For further clarification of the picture, the two grasses were analyzed separately in a randomized block design. The data are given in Tables 11 and 12 for Pangolagrass and Bahiagrass respectively. No significant differences were found between fertility levels in Pangolagrass, but the opposite was true for Bahiagrass. From Figure 12 one observes that by far the greatest degree of hardiness was shown at this time by Bahiagrass at the 400 pound level of fertility. The indications from the graph are that somewhat the same relationship existed between the 100 pound level of Bahiagrass and the 400 pound level of Pangolagrass as existed in the first period. It would appear that the difference in the

TABLE 9

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF THE TWO GRASSES FOR THE FIRST PERIOD DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Grasses | 1 | 8,367.56 | 8,367.56* |
| Replications | 2 | 1,192.52 | 596.26 |
| Error (a) | 2 | 609.82 | 304.91 |
| Subplots: | | | |
| Fertility levels | 1 | 3,758.43 | 3,758.43* |
| F x G | 1 | 94.08 | 94.08 |
| Error (b) | 4 | 1,938.88 | 484.72 |
| Samples | 60 | 3,283.88 | 54.73 |
| Total | 71 | 19,245.18 | |

*Significant at the 5 per cent level.

TABLE 10

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF THE TWO GRASSES FOR THE SECOND PERIOD DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Grasses | 1 | 5,156.20 | 5,156.20* |
| Replications | 2 | 3,233.34 | 1,616.67 |
| Error (a) | 2 | 265.81 | 132.91 |
| Subplots: | | | |
| Fertility levels | 1 | 4,534.69 | 4,534.69** |
| F x G | 1 | 2,580.02 | 2,580.02** |
| Error (b) | 4 | 393.53 | 98.38 |
| Samples | 60 | 1,630.27 | 27.17 |
| Total | 71 | 17,832.58 | |

*Significant at the 5 per cent level.
**Significant at the 1 per cent level.

TABLE 11

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF PANGOLAGRASS FOR THE SECOND PERIOD AS AFFECTED BY FERTILITY TREATMENT DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 5 | 402.47 | |
| Fertility levels | 1 | 23.20 | 23.20 |
| Replications | 2 | 325.75 | 162.88 |
| Error | 2 | 53.62 | 26.72 |

TABLE 12

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF BAHIAGRASS FOR THE SECOND PERIOD AS AFFECTED BY FERTILITY DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Squares |
|---|---|---|---|
| Total | 5 | 1,659.25 | |
| Fertility levels | 1 | 1,317.20 | 1,317.20** |
| Replications | 2 | 340.43 | 170.22** |
| Error | 2 | 1.62 | 0.81 |

**Significant at the 1 per cent level.

degree of cold hardiness which the graph shows for the two levels of Pangolagrass during the first two observation dates of this period is offset by the seeming inconsistency which occurred on the third observation date when the 400 pound level gave indications of being the more hardy of the two. A probably reason for this reversal of form in Pangolagrass is that this third observation date followed a period of lower temperatures as may be seen in Figure 11.

The graph in Figure 12 indicates that during the third period differences were not so great as in the previous two periods. That this is true is borne out by the data given in Table 13. It is to be noted here that there were no differences between the two grasses in hardiness at this time. There was, however, no interaction between grasses and fertility levels. When the two grasses were analyzed separately as in the above case, further emphasis was given to the condition of no difference. These data appear in Tables 14 and 15. Although not supported statistically, the graph shows that Bahiagrass still appeared to have the same relationship between the two nitrogen levels but to a lesser extent.

The statistical analysis for the fourth period is given in Table 16. As the graph in Figure 13 indicates there was a difference in the cold hardiness of the two grasses in the fourth period. Bahiagrass was in possession of the greater degree of cold hardiness in this period.

TABLE 13

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF THE TWO GRASSES FOR THE THIRD PERIOD DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Grasses | 1 | 3,722.87 | 3,722.87 |
| Replications | 2 | 707.56 | 353.78 |
| Error (a) | 2 | 1,086.44 | 543.22 |
| Subplots: | | | |
| Fertility levels | 1 | 506.13 | 506.13 |
| F x G | 1 | 1,872.22 | 1,872.22* |
| Error (b) | 4 | 322.40 | 80.60 |
| Samples | 60 | 1,825.11 | 30.42 |
| Total | 71 | 14,363.32 | |

*Significant at the 5 per cent level.

TABLE 14

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF BAHIAGRASS FOR THE THIRD PERIOD AS AFFECTED BY FERTILITY TREATMENT DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 5 | 289.34 | |
| Fertility levels | 1 | 248.67 | 248.67 |
| Replications | 2 | 7.11 | 3.56 |
| Error | 2 | 33.56 | 16.78 |

## TABLE 18

## ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF PANGOLAGRASS FOR THE THIRD PERIOD AS AFFECTED BY FERTILITY TREATMENT DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 5 | 639.22 | |
| Fertility levels | 1 | 45.93 | 45.93 |
| Replications | 2 | 529.27 | 264.64 |
| Error | 2 | 64.02 | 32.01 |

## TABLE 19

## ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF THE TWO GRASSES FOR THE FOURTH PERIOD DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Grasses | 1 | 7,725.84 | 7,784.24* |
| Replications | 2 | 1,927.69 | 963.84 |
| Error (a) | 2 | 348.19 | 174.09 |
| Subplots | | | |
| Fertility levels | 1 | 9.54 | 9.54 |
| F x G | 1 | 941.78 | 941.78* |
| Error (b) | 4 | 228.90 | 57.22 |
| Samples | 60 | 2,383.25 | 39.72 |
| Total | 71 | 13,958.60 | |

*Significant at the 5 per cent level.

No difference between fertility levels is indicated by the data in Table 16, but an interaction between grasses and fertility levels is noted. The separate analyses of the two grasses are given in Tables 17 and 18. From these one notes a difference in cold hardiness between the two fertility levels in Pangolagrass but not in Bahiagrass. The graph in Figure 12 shows that greater cold hardiness was exhibited at the 100 pound level in Pangolagrass. Except for the second observation date of this period, it would appear from this graph that the pattern previously established for Bahiagrass was still holding.

TABLE 17

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF PANGOLAGRASS FOR THE FOURTH PERIOD AS AFFECTED BY FERTILITY TREATMENT DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 5 | 307.36 | |
| Fertility levels | 1 | 86.64 | 86.64* |
| Replications | 2 | 214.66 | 107.33* |
| Error | 2 | 5.67 | 2.84 |

*Significant at 5 per cent level.

Data for the analysis of the fifth period are given in Table 18. The data in this table indicate that there was a difference in the hardiness of the two grasses and in the

TABLE 18

ANALYSES OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF BAHIAGRASS FOR THE FOURTH PERIOD AS AFFECTED BY FERTILITY TREATMENT DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Total | 5 | 247.49 | |
| Fertility levels | 1 | 64.68 | 64.68 |
| Replications | 2 | 156.59 | 78.30 |
| Error | 2 | 26.22 | 13.11 |

TABLE 19

ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF THE TWO GRASSES FOR THE FIFTH PERIOD DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Grasses | 1 | 39,649.28 | 39,649.28** |
| Replications | 2 | 1,657.70 | 828.85 |
| Error (a) | 2 | 468.91 | 234.46 |
| Subplots: | | | |
| Fertility levels | 1 | 1,339.90 | 1,339.90** |
| F x G | 1 | 8.32 | 8.32 |
| Error (b) | 4 | 170.27 | 42.57 |
| Samples | 60 | 988.57 | 12.64 |
| Total | 71 | 44,284.68 | |

**Significant at the 1 per cent level.

cold hardiness of the separate fertility levels. Since there was no significant interaction, the grasses were not analyzed separately. From the graph in Figure 12 one notes that greatest cold hardiness was found in Bahiagrass. In both grasses at this time, the higher degree of cold hardiness existed at the 400 pound level of nitrogen. This was the prevalent condition for Bahiagrass but appeared to be true of Pangolagrass only following periods of warm temperatures.

Table 20 contains the statistical analysis for the sixth period. No statistically significant differences existed between any of the variable under consideration during that period, if the period is considered as a whole. However, the graph in Figure 12 does indicate that a considerable amount of variation did exist on the various dates of observation. On each observation date of the sixth period, Bahiagrass was more hardy at the 400 pound level of nitrogen. Pangolagrass appeared more hardy at the 400 pound level only on the first date of observation in the sixth period. It is interesting to note that on the first date of observation there was only a very slight difference between the cold hardiness indicated for the two levels of fertilization in Bahiagrass.

Yield data and estimations of winter injury in 1958-59 are given in Table 4. These data indicate that Pangolagrass was more severely injured during the winter of 1958-59 than Pensacola Bahiagrass. An interesting comparison can be made between these data and those for the 1957-58 season. In making this comparison, one notes that in the earlier year Pangolagrass was much more severely injured than in 1958-59 but that the opposite is true of Bahiagrass. It is to be noted, however, that in neither year was Bahiagrass damaged to any great extent.

Yield data show that injury was probably greatest at the 400 pound level in Pangolagrass. Little if any difference in hardiness can be distinguished between the two levels of fertility in Bahiagrass if the ratios of the yields at the two levels for the season total in 1958 and for the first cutting in 1959 are compared. Since the 1958 season-total yields ratio is 1:2.4 and the first cutting of 1959 yields ratio is 1:2.9, there is some basis for stating that possibly the grass at the 400 pound level was less severely damaged than that at the 100 pound level.

In all cases one notes that yields were greater at the higher level of nitrogen. The nitrogen content of the forage was also higher at the 400 pound level of nitrogen in all cases. These are expected results; however, the narrowness of the nitrogen content difference between the two fertility levels in Bahiagrass is somewhat unexpected.

## TABLE 20

### ANALYSIS OF VARIANCE: PER CENT OF TOTAL CONDUCTIVITIES OF THE TWO GRASSES FOR THE SIXTH PERIOD DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Grasses | 1 | 17,612.56 | 17,612.56 |
| Replications | 2 | 5,980.49 | 2,990.25 |
| Error (a) | 2 | 2,356.24 | 1,178.62 |
| Subplots: | | | |
| Fertility level | 1 | 965.80 | 965.80 |
| F x G | 1 | 22.12 | 80.12 |
| Error (b) | 4 | 3,391.61 | 847.90 |
| Samples | 60 | 1,048.48 | 17.48 |
| Total | 71 | 31,348.31 | |

Nitrogen in samples

The nitrogen content of the material from which samples were taken for the conductivity experiments was determined for each observation date of the last five periods. These data are summarized in Tables 6 and 7. Utilizing periods as main plots and grasses as subplots with fertility levels being sub-subplots, an analysis of the five periods is given in Table 21. From these data one can determine that there was a difference in the nitrogen content of the two grasses at different periods but no significant difference in the overall content of the two grasses. There was an interaction between grasses and periods. The highly significant difference between fertility levels needs to be considered in the light of the interaction between fertility levels and grasses. For this reason, a separate analysis of the two grasses is given in Tables 22 and 23. Here one observes that in each grass there was a real difference between periods as to the nitrogen content and also a difference between fertility levels as to nitrogen content. It is to be noted in Tables 7 and 8 that, except for the December 16 sampling in Bahiagrass, all nitrogen contents are higher at the 400 pound level of fertility. Also to be noted is that only on the sampling dates of February 24 and March 16 did a higher nitrogen content occur at the 100 pound level in Pangolagrass.

The data in Table 24 is presented in an attempt to correlate the nitrogen content of the various grass samples with their hardiness, as indicated by per cent of total conductivity. In no case was there a correlation. Only in Bahiagrass at the 100 pound level did the correlation coefficient even approach significance. At this fertility level Bahiagrass demonstrated its lowest degree of cold hardiness.

### Summary of 1958-59 experiment

Studies of cold hardiness in Pensacola Bahiagrass and Pangolagrass at fertility rates of 100 and 400 pounds

TABLE 21

ANALYSIS OF VARIANCE: NITROGEN CONTENT OF SAMPLES FROM EACH OF TWO GRASSES IN FIVE SEPARATE PERIODS AS AFFECTED BY FERTILITY TREATMENT DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Replications | 2 | 0.31 | 0.16 |
| Periods | 4 | 7.06 | 1.76** |
| Error (a) | 8 | 0.66 | 0.08 |
| Subplots: | | | |
| Grass | 1 | 0.11 | 0.11 |
| G x P | 4 | 2.20 | 0.55* |
| Error (b) | 10 | 1.59 | 0.16 |
| Sub-subplots: | | | |
| Fertility levels | 1 | 8.86 | 8.86** |
| F x P | 4 | 0.70 | 0.18 |
| F x G | 1 | 0.61 | 0.61** |
| F x G x P | 4 | 0.86 | 0.22 |
| Error (c) | 20 | 1.98 | 0.10 |
| Total | 59 | 24.03 | |

**Significant at the 1 per cent level.

*Significant at the 5 per cent level.

## TABLE 22

### ANALYSIS OF VARIANCE: NITROGEN CONTENT OF SAMPLES FROM PANGOLAGRASS IN FIVE SEPARATE PERIODS AS AFFECTED BY FERTILITY TREATMENT DURING THE 1958-59 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Periods | 4 | 6.90 | 1.73** |
| Replications | 2 | 0.83 | 0.42 |
| Error (a) | 8 | 0.78 | 0.10 |
| Subplots: | | | |
| Fertility levels | 1 | 1.69 | 1.69** |
| F x P | 4 | 0.50 | 0.13 |
| Error (b) | 10 | 1.10 | 0.11 |
| Total | 29 | 11.81 | |

**Significant at the 1 per cent level.

## TABLE 23

### ANALYSIS OF VARIANCE: NITROGEN CONTENT OF SAMPLES FROM BAHIAGRASS IN FIVE SEPARATE PERIODS AS AFFECTED BY FERTILITY TREATMENT DURING THE 1959-60 SEASON

| Source | D.F. | Sum of Squares | Mean Square |
|---|---|---|---|
| Main Plots: | | | |
| Periods | 4 | 3.31 | 0.83** |
| Replications | 2 | 0.35 | 0.18 |
| Error (a) | 8 | 0.58 | 0.07 |
| Subplots: | | | |
| Fertility levels | 1 | 5.77 | 5.77** |
| F x P | 4 | 1.21 | 0.30 |
| Error (b) | 10 | 0.88 | 0.09 |
| Total | 29 | 12.10 | |

**Significant at the 1 per cent level.

of nitrogen per acre per year were conducted. These studies utilized the modified electrical conductivity methods, advanced by Dexter (22), in which per cent of total conductivity is obtained. A statistical analysis was made of the data obtained. Climatic conditions which existed during the experiment were measured and presented. Differences in the severity of the weather during the 1957-58 season and the 1958-59 season were noted. Yield data were obtained and related to winter injury. Nitrogen content was determined for the samples on which cold hardiness tests were run. Comparisons were made of the results obtained in the 1957-58 and those obtained in 1958-59.

## TABLE 24

## CORRELATION COEFFICIENTS FOR THE CORRELATION OF NITROGEN CONTENT AND PER CENT OF TOTAL CONDUCTIVITY IN PENSACOLA BAHIAGRASS AND PANGOLAGRASS AT THE 100 AND 400 POUND LEVELS OF NITROGEN PER ACRE PER YEAR

| Grass | Fertility Level | r |
|---|---|---|
| Pensacola Bahiagrass | 100 lbs. N | -0.477 |
| Pensacola Bahiagrass | 400 lbs. N | -0.093 |
| Pangolagrass | 100 lbs. N | 0.202 |
| Pangolagrass | 400 lbs. N | 0.061 |

In comparing the results obtained in 1958 with those of 1957, indications were that for Bahiagrass, cold hardiness was generally greater at the 400 pound level of nitrogen during both years. Pangolagrass appeared to exhibit greater cold hardiness at the 100 pound level during both years.

Conductivity measurements show that apparently both grasses were more cold hardy during the 1957 season than in 1958-59. This must be considered in the light of the fact that climatic conditions were very much more severe during the earlier winter and, in spite of the degree of cold hardiness exhibited, damage, especially to Pangolagrass, during the winter of 1957 was more extensive. In April 1959, some indications were that Bahiagrass was more damaged in 1958-59.

Utilization of values of per cent of total conductivity as indicators of cold hardiness allowed more faith to be placed in comparisons of cold hardiness between fertility levels and between grasses than when specific conductivity readings alone were used.

Periodic differences in cold hardiness were observed to exist in both grasses at both fertility levels. Greater fluctuation between fertility levels on 80 degree of cold hardiness was noted in Pangolagrass. Wider spreads in degree of cold hardiness were more prevalent in Bahiagrass, but the 100 pound level of nitrogen consistently produced the more cold hardy tissue in Bahiagrass.

Pangolagrass was more severely injured during the winter of 1958-59 than Bahiagrass. Yield data obtained from the first cutting in 1959 show that greater injury was probably suffered by Pangolagrass at the 400 pound level of nitrogen while little difference in the amount of injury could be detected between the two levels in Bahiagrass insofar as yields indicated.

Greater amounts of nitrogen were found to be present in the samples from grass fertilized with 400 pounds of nitrogen per acre per year. This is the expected result.

An attempt was made to correlate nitrogen content with degree of cold hardiness. In no case was there any correlation.

## TEMPERATURE AND COLD HARDINESS

Various research workers (40, 69) have reported that grasses develop cold hardiness upon exposure to low temperatures and might lose this hardiness after a period of higher temperatures. An attempt has been made in this study to determine whether there was any correlation between temperatures and the cold hardiness as measured by per cent of total conductivity. Data for these analyses are given in Tables 25 and 26. In the first of these two tables the correlation coefficients for hardiness with average minimum temperatures are given. The average minimum temperatures used are those which occurred between the various observation periods. In the case of the first observation period, the average for the seven preceding days is used. A correlation could only be found between these temperatures and the hardiness of Pangolagrass at the 400 pound level of nitrogen. The correlation in this case was negative as would be expected. Since the picture given by the lack of correlation of the other groups with minimum temperatures seemed to indicate that further consideration of the problem was necessary, an attempt was also made to correlate hardiness with the average mean temperatures occurring between the observation dates. These data are given in Table 26 and show that no significant

### TABLE 25

### CORRELATION COEFFICIENTS FOR THE CORRELATION OF TEMPERATURE WITH PER CENT OF TOTAL CONDUCTIVITY IN PENSACOLA BAHIAGRASS AND PANGOLAGRASS AT THE 100 AND 400 POUND LEVELS OF NITROGEN PER ACRE PER YEAR

| Grass | Fertility Level in Lbs. of N | r for correlation with Average Minimum | Average Mean | Low Minimum |
|---|---|---|---|---|
| Pensacola Bahiagrass | 100 | 0.210 | 0.276 | 0.239 |
| Pensacola Bahiagrass | 400 | -0.048 | 0.010 | -0.004 |
| Pangolagrass | 100 | 0.141 | 0.141 | 0.143 |
| Pangolagrass | 400 | -0.487* | -0.418 | -0.463* |

*Significant at the 5 per cent level.

### TABLE 26

### TEMPERATURES USED IN DETERMINING CORRELATIONS OF TEMPERATURE WITH HARDINESS

| Observation Date | Average Minimum Since the last Observation Date | Average Mean Since the Last Observation Date | Lowest Minimum Since the Last Observation Date |
|---|---|---|---|
| Sept. 17 | 70°F. | 80.4°F. | 65°F. |
| Sept. 23 | 71 | 81.7 | 68 |
| Sept. 30 | 69 | 78.4 | 58 |
| Oct. 14 | 63 | 70.1 | 58 |
| Oct. 23 | 61 | 68.4 | 54 |
| Nov. 4 | 61 | 63.6 | 40 |
| Nov. 18 | 53 | 66.6 | 47 |
| Nov. 25 | 55 | 66.1 | 37 |
| Dec. 6 | 56 | 65.6 | 40 |
| Dec. 9 | 63 | 63.1 | 40 |
| Dec. 16 | 41 | 58.0 | 28 |
| Jan. 6 | 44 | 60.1 | 28 |
| Feb. 10 | 44 | 67.6 | 36 |
| Feb. 17 | 61 | 71.6 | 38 |
| Feb. 24 | 47 | 60.3 | 33 |
| Mar. 3 | 53 | 61.6 | 41 |
| Mar. 16 | 40 | 60.0 | 33 |
| Mar. 18 | 45 | 67.0 | 32 |

correlation exists; however, the correlation coefficient for Pangolagrass at the 400 pound level approaches significance.

A third attack was made on the correlation of cold hardiness with temperature when the lowest minimum temperature which occurred between the conductivity readings was used. The results are summarized in Tables 25 and 26. A correlation existed between the lowest minimum temperatures and the hardiness of Pangolagrass at the 400 pound level of nitrogen.

## Microclimate Investigations

A parallel study of microclimatic conditions was undertaken in conjunction with the hardiness study in order that some idea of the conditions of temperature which actually exist in the area near the plants being studied might be had. Previous work of this nature has been cited (79) in which Sprague and his associates studied variations in the air temperatures of the microclimate. They found the greatest extremes in minimum temperatures to exist near the ground level.

Throughout the present study, the lowest nocturnal temperatures obtained were those taken at a height of three inches above the ground surface. This phenomenon is completely in line with that of nocturnal temperature inversion which has been extensively discussed by Geiger (27).

Data substantiating this claim are given in Table 27. The data given in this table have been selected at random, but they present essentially the same picture as other data obtained.

TABLE 27

AVERAGE TEMPERATURES FOR THE FOURTEEN-HOUR PERIOD FROM 6:00 P.M. UNTIL 8:00 A.M. ON CERTAIN SPECIFIED DATES FROM PLOTS OF BAHIAGRASS, PANGOLAGRASS, AND BERMUDAGRASS

| Date | Grass | Five Feet Level | Three Inch Level | Soil Surface |
|---|---|---|---|---|
| Dec. 15, 1955 | Bahiagrass (1.5" tall) | 30.4°F. | 28.9°F. | 33.5°F. |
| Jan. 17, 1959 | Bahiagrass (1.5" tall) | - | 28.5 | 35.5 |
| Jan. 22, 1955 | Pangolagrass (Dead, 8" tall) | 35.5 | 30.5 | 37.2 |
| Dec. 22, 1955 | Pangolagrass (Dead, 8" tall) | 33.5 | 32.5 | 38.9 |
| Dec. 1, 1957 | Bermudagrass (5" tall) | 30.3 | 27.4 | 42.4 |
| Dec. 12, 1957 | Bare Soil (Beside grass) | 11.5 | 9.5 | - |
| Oct. 27, 1957 | Pangolagrass (Short, 3½") | 39.9 | 33.5 | 46.4 |
| Oct. 27, 1957 | Bahiagrass (Medium, 9") | 39.0 | 35.5 | 46.5 |
| Feb. 15, 1958 | Bermudagrass (Dead, 5" tall) | 25.7 | 21.4 | 34.9 |

Also to be seen from the data in Table 27 is the fact that the temperature of the soil surface remained considerably warmer than the temperatures at either the five-foot level or those at the three-inch level. This was a consistent occurrence.

Hourly nocturnal temperatures for three rather cool nights are given in Tables 28, 29, and 30. In this first table, the data for the night of October 27, 1957 are recorded. The occurrence of freezing temperatures this early in the fall was a record-setting event for the district. Less wind movement was recorded for this night than for the two nights for which data are presented in Tables 29 and 30. In a comparison of the temperatures at the three-inch level with those at the five-foot level, in the three tables, one notes that under conditions of less wind there is a greater variation between the two levels. For example, on the less windy night of October 27, the average temperature at the five-foot level was 28.6° F. while at the three-inch level the average temperature was 22.2° F. in Bahiagrass and 35.3° F. in Pangolagrass. Differences of 7.1° and 4.3° respectively occur here. On the more windy nights of January 12 and January 22 differences between the five-foot level and the three-inch level are 1.8° and 2.6° respectively for Bahiagrass and Pangolagrass. This is in agreement with the phenomenon outlined by Geiger (27) in which an increase in wind movement will act to destroy the temperature stratification in which a cold layer has been built up near the ground.

## TABLE 28

## AIR TEMPERATURES IN DEGREES FAHRENHEIT IN A PLOT OF SHORT PANGOLAGRASS AND IN A PLOT OF MEDIUM HIGH BAHIAGRASS ON THE NIGHT OF OCTOBER 27, 1957

| Time | Pangolagrass<br>Five Feet Level | Pangolagrass<br>Three Inch Level | Pangolagrass<br>Soil Surface | Bahiagrass<br>Five Feet Level | Bahiagrass<br>Three Inch Level | Bahiagrass<br>Soil Surface |
|---|---|---|---|---|---|---|
| | Degrees Fahrenheit | | | | | |
| 6:00 P.M. | 54.5 | 46.0 | 55.5 | 54.5 | 41.5 | 58.5 |
| 7:00 P.M. | 49.5 | 33.0 | 56.0 | 48.5 | 38.5 | 48.5 |
| 8:00 P.M. | 43.5 | 33.5 | 50.0 | 43.5 | 38.5 | 48.5 |
| 9:00 P.M. | 38.5 | 31.5 | 48.0 | 38.5 | 35.5 | 48.5 |
| 10:00 P.M. | 35.5 | 33.5 | 48.5 | 39.5 | 36.5 | 48.5 |
| 11:00 P.M. | 38.5 | 33.0 | 48.5 | 38.5 | 36.0 | 48.5 |
| 12:00 P.M. | 38.5 | 31.0 | 46.5 | 38.5 | 35.5 | 48.5 |
| 1:00 A.M. | 36.5 | 36.5 | 46.5 | 35.0 | 35.5 | 48.0 |
| 2:00 A.M. | 38.5 | 32.0 | 46.5 | 39.5 | 35.5 | 48.5 |
| 3:38 A.M. | 37.5 | 32.5 | 44.5 | 37.5 | 34.5 | 46.5 |
| 4:00 A.M. | 36.0 | 31.5 | 43.5 | 36.5 | 33.5 | 44.5 |
| 5:00 A.M. | 34.0 | 30.5 | 48.5 | 34.5 | 35.5 | 43.0 |
| 6:00 A.M. | 33.5 | 30.5 | 46.5 | 33.0 | 31.5 | 43.0 |
| 7:00 A.M. | 34.5 | 31.5 | 48.5 | 34.5 | 32.5 | 48.5 |
| Average | 39.5 | 32.5 | 48.4 | 30.5 | 35.3 | 48.0 |

Temperature ranges for various dates are given in Table 31. These data indicate that the wider range in temperatures occurred at the three-inch and at the twelve-inch levels with more narrow ranges at the five-feet level and at the soil surface. A prime example is the first one listed in the table in which the ranges were 38.5°, 50.6°, 48.5°, 42.0° for the five-feet level, the twelve-inch level, the three-inch level, and the soil surface respectively.

TABLE 29

AIR TEMPERATURES IN DEGREES FAHRENHEIT IN A PLOT OF SHORT PANGOLAGRASS ON THE NIGHT OF JANUARY 30, 1958

| Time | Five Foot Level | Twelve Inch Level | Three Inch Level | Surface of Soil |
|---|---|---|---|---|
| | Degrees Fahrenheit | | | |
| 6:30 P.M. | 46.5 | 44.5 | 41.5 | 45.5 |
| 7:30 P.M. | 42.0 | 41.5 | 39.5 | 43.5 |
| 8:00 P.M. | 41.5 | 40.5 | 39.0 | 42.5 |
| 9:00 P.M. | 35.0 | 35.5 | 34.0 | 40.5 |
| 10:00 P.M. | 36.5 | 36.5 | 33.0 | 39.5 |
| 11:00 P.M. | 34.5 | 31.5 | 31.0 | 38.5 |
| 12:00 P.M. | 30.5 | 32.0 | 31.5 | 37.5 |
| 1:00 A.M. | 30.5 | 30.0 | 27.5 | 36.0 |
| 2:00 A.M. | 30.5 | 29.5 | 28.5 | 35.0 |
| 3:00 A.M. | 29.5 | 27.5 | 27.0 | 34.5 |
| 4:00 A.M. | 27.0 | 25.5 | 25.5 | 33.5 |
| 5:00 A.M. | 27.5 | 25.5 | 23.5 | 33.5 |
| 6:00 A.M. | 25.5 | 27.0 | 26.5 | 33.5 |
| 7:00 A.M. | 26.0 | 26.5 | 26.5 | 31.0 |
| Average | 33.5 | 35.1 | 30.5 | 37.5 |

The findings of Sprague et al. (79) appear to be in good agreement with those of the present study. During the winter months, they found mean daily temperature ranges to be wider at the three-inch level than at the five-foot level in Maryland and New Jersey and to a smaller extent in Pennsylvania, while the pattern was inconsistent under snow cover in Vermont.

It is interesting to observe in Table 31 that, even on days when very low minimum temperatures were recorded, daytime maximum temperatures often were very high. The effect of these high maximum temperatures on the maintenance of high degrees of hardiness is a problem which definitely needs to be considered in hardiness studies.

TABLE 30

AIR TEMPERATURES IN DEGREES FAHRENHEIT IN A PLOT OF SHORT BAHIAGRASS ON THE NIGHT OF JANUARY 16, 1955

| Time | Five Foot Level | Twelve Inch Level | Three Inch Level | Surface of Soil |
|---|---|---|---|---|
| | Degrees Fahrenheit | | | |
| 6:00 P.M. | 43.0 | 42.0 | 41.0 | 42.0 |
| 7:00 P.M. | 40.5 | 35.0 | 38.0 | 40.0 |
| 8:00 P.M. | 35.5 | 35.0 | 35.0 | 38.0 |
| 9:00 P.M. | 36.5 | 35.0 | 32.5 | 37.0 |
| 10:00 P.M. | 34.5 | 33.5 | 33.0 | 38.0 |
| 11:00 P.M. | 31.5 | 31.5 | 30.5 | 33.0 |
| 12:00 P.M. | 30.5 | 28.5 | 28.0 | 33.0 |
| 1:00 A.M. | 28.5 | 28.0 | 27.0 | 32.0 |
| 2:00 A.M. | 26.5 | 27.5 | 26.0 | 31.5 |
| 3:00 A.M. | 26.5 | 24.5 | 24.0 | 30.0 |
| 4:00 A.M. | 25.5 | 24.0 | 24.0 | 25.5 |
| 5:00 A.M. | 24.5 | 23.0 | 23.0 | 29.5 |
| 6:00 A.M. | 23.0 | 22.5 | 22.5 | 28.5 |
| 7:00 A.M. | 22.5 | 21.0 | 21.0 | 28.0 |
| Average | 30.4 | 28.5 | 28.6 | 33.5 |

Summary of Temperature Studies

Temperature data were obtained from measurements made at the five-foot level with maximum and minimum thermometers in a standard weather station located near the experimental plots. These data were used in attempts to correlate temperature with hardiness. Included in this correlation study were average minimum temperature which occurred between the various observation dates, the average mean temperature which occurred between the observation

TABLE 61

TEMPERATURE RANGES ON VARIOUS DATES CHOSEN AT RANDOM FROM THE MICROCLIMATE STUDIES

| Date | Grass or Soil | Degrees Fahrenheit<br>5 Foot Level<br>Max. | <br><br>Min. | <br><br>Diff. | <br>12 Inch Level<br>Max. | <br><br>Min. | <br><br>Diff. | <br>3 Inch Level<br>Max. | <br><br>Min. | <br><br>Diff. | <br>Soil Surface<br>Max. | <br><br>Min. | <br><br>Diff. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dec.2,1957 | Bare Soil | 54.0 | 18.0 | 36.0 | 72.0 | 36.0 | 36.0 | 63.0 | 18.0 | 45.0 | 78.0 | 30.0 | 48.0 |
| Dec.5,1957 | Bermuda 3" tall | 68.0 | 30.0 | 38.0 | - | - | - | 58.0 | 24.0 | 34.0 | 73.5 | 19.5 | 54.0 |
| Dec.10,1957 | Bahia 6" tall | 59.0 | 32.0 | 27.0 | 54.0 | 31.0 | 23.0 | 61.0 | 30.0 | 31.0 | 53.5 | 41.5 | 12.0 |
| Dec.12,1957 | Bare Soil | - | - | - | 37.0 | 13.0 | 24.0 | 46.0 | 18.0 | 28.0 | - | - | - |
| Dec.12,1957 | Bahia 8" tall | - | - | - | 37.0 | 12.0 | 25.0 | 50.0 | 7.0 | 43.0 | 42.0 | 30.0 | 12.0 |
| Dec.12,1957 | Bermuda 3" tall | - | - | - | - | - | - | - | - | - | 50.0 | 26.0 | 24.0 |
| Jan.8,1958 | Bare Soil | 44.0 | 18.0 | 26.0 | 48.0 | 12.0 | 36.0 | 45.0 | 13.0 | 32.0 | 43.0 | 27.5 | 15.5 |
| Feb.4,1958 | Bare Soil | 54.0 | 22.0 | 32.0 | 57.5 | 19.5 | 38.0 | 58.0 | 20.0 | 38.0 | 67.0 | 26.0 | 41.0 |
| Feb.4,1958 | Bahia 6" tall | 54.0 | 22.0 | 32.0 | 58.0 | 22.0 | 36.0 | 61.0 | 16.0 | 45.0 | 54.0 | 32.0 | 22.0 |
| Feb.4,1958 | Bermuda 3" tall | 54.0 | 22.0 | 32.0 | - | - | - | 48.0 | 24.0 | 24.0 | - | - | - |
| Feb.10,1958 | Bare Soil | 47.0 | 27.0 | 20.0 | 48.0 | 22.0 | 26.0 | 58.0 | 22.0 | 36.0 | 56.0 | 33.0 | 23.0 |
| Feb.10,1958 | Bermuda 6" tall | 47.0 | 27.0 | 20.0 | 48.0 | 23.0 | 25.0 | 53.0 | 22.0 | 31.0 | 51.0 | 34.0 | 17.0 |

dates, and the lowest minimum temperature which occurred between the observation dates.

Microclimate investigations involved the taking of temperature data in the grass plots with a sixteen-point recording potentiometer utilizing copper-constantan thermocouples. Vertical levels of measurement included soil surface, three inches above the surface, twelve inches above the surface, and five feet above the surface.

No correlations were observed between hardiness and the temperatures used except between the minimum temperatures, both the low and the average, and the hardiness of Pangolagrass at the 400 pound level of nitrogen.

The microclimate investigations have revealed that the coldest temperatures observed in the study were found three inches above the soil surface on winter nights. They have also shown that the soil surface under a grass cover remained considerably warmer than the air above it. Wind was observed to reduce temperature stratification. Temperature ranges were found to be greater at the three-inch level and at the twelve-inch level than at either the five-foot level or at soil surface. Nights in which rather low minimum temperatures occurred were often followed by days with high maximum temperatures. Notation is made of the fact that good general agreement is found between temperature phenomena observed in this study and those observed by other investigators.

# DISCUSSION

From observations made during both the 1957-58 season and the 1958-59 season, it would appear that Pensacola Bahiagrass is more hardy than Pangolagrass under either the 100 or 400 pound rate of nitrogen fertilization. This would generally be the expected result in that Pangolagrass has been observed to be less adapted to cold weather (35).

The microclimate investigations of this study have borne out the fact of the occurrence of a nocturnal inversion of temperature. This phenomenon has been pointed out by Geiger (27). These investigations have shown that soil surface temperatures were several degrees warmer than the air temperature. This indicates that the parts of the plants in contact with the air layer just above the soil surface are subject to the more severe temperatures. In view of this, one can see why Bahiagrass, with a system of stems located at and below the soil surface is subjected to less severe cold weather than Pangolagrass whose stems are at or above the soil surface. When this advantage is coupled with the greater degree of hardiness also exhibited by Bahiagrass, it becomes quite clear how Pangolagrass can be heavily winter-killed while Bahiagrass is only slightly damaged. McCloud and Creel (50) have stated that successive growth periods followed by freezes will cause winter killing of Pangolagrass and observed that this is due to a lack of root reserves.

Bahiagrass gave indications of being more hardy at the higher level of nitrogen. However, following periods when temperatures were low the difference between hardiness in Bahiagrass at the two fertility levels became more narrow.

Blackman (3) noted that no growth in pastures took place below a soil temperature of 42° F. Between 42° and 47° F. "nitrogen-manured" pastures were observed to grow at a greater rate than his control. Continued growth is thought to be a deterrent to hardiness. The tendency of a highly fertilized grass to continue growth when grass at a lower fertility level was not growing might tend to equalize the degrees of hardiness held by both, if the former had previously been the more hardy. This was evidently the case as is shown by the fact that, following periods of low temperatures, the hardiness exhibited by the grass at the two levels of nitrogen tended toward an equilibrium.

Indications were that during most of the season there was only a small degree of hardiness at either fertility level in Bahiagrass and that conditions suitable for producing any degree of hardening did not exist. The presence of long periods of warm weather during the winter caused losses of hardiness. Dexter (19) has suggested that an efficient cold-temperature hardening reaction requires retarded vegetative growth. The warm periods interspersed with the cold caused resumption of vegetative growth and reduced the efficiency of the hardening process. The ability of the plants under the higher rate of nitrogen to withstand the cold weather that did occur was probably attributable to their greater food reserves and their better general condition. The much larger stems present at the higher fertility level give evidence of the better condition of the grass. During the 1957-58 season, the hardiness of the two levels appeared to be developing in opposite directions during cold weather. There was a trend which indicated that the plants at the higher level of nitrogen appeared to be less hardy than those at the lower level. This could be compared to the findings of Carroll and Welton (9) in which they observed that no difference in the hardiness of Kentucky Bluegrass at high and low levels of nitrogen existed in the unhardened condition, but that, in the hardened condition, the plants under the higher fertility level were less hardy than those under the lower level.

Conductivity measurements showed that Bahiagrass was more hardy at the higher fertility level during most of both seasons. These findings were backed by survival studies in the greenhouse in 1957. Yield data and estimations of winter injury indicated that no real differences existed between the grass grown at the two fertility levels insofar as ability to survive the winter was concerned.

These results showing the greater hardiness at the higher fertility level become particularly interesting when they are viewed in the light of such experiments as those of Creel (15), who found winter killing of Pangolagrass to increase with increased applications of nitrogen; Edgerton (23), who found nitrogen to lower the hardiness of apple trees when applied in the fall; Kimball (37), who found greater tenderness among herbaceous plants treated with sodium nitrate; and Levitt (41), who has observed that nitrogen decreases hardiness of plants in general.

Not all workers, however, have found nitrogen to decrease hardiness. Magee (54) found no difference in hardiness between naturally hardened alfalfa roots which were fertilized with 0-8-24 in late May, those fertilized with nitrate of soda in late September, or his unfertilized controls.

No correlation could be found in the present study between hardiness and the total nitrogen content of the samples being tested for hardiness. This was true for both grasses at both fertility levels. As expected, the samples from the grass grown at the higher rates of nitrogen did contain the larger amounts of nitrogen.

Greathouse and Stuart (29) found in their study of red clover roots that winter hardiness was associated with a greater concentration of nitrogen. Carroll and Welton (9) observed that Bluegrass fertilized with heavy and late applications of nitrogen had a higher nitrogen content but, after a hardening period, was less hardy than grass fertilized at lower rates and which possessed a lower content of nitrogen.

Levitt (42) states that the efforts to relate hardiness to total or protein nitrogen were doomed to failure in that total nitrogen can be increased by nitrogen fertilization while this in itself reduces frost hardiness. The results obtained in this study both agree and contrast with the opinion expressed by Levitt. The results obtained with Bahiagrass at the 400 pound level indicate that the general statement that nitrogen reduced hardiness might be made with reservations.

Yields of both Bahiagrass and Pangolagrass were greater at the higher level of nitrogen fertilization. These findings agree with those of such investigators as Blue and Gammon (5), who found that when they applied nitrogen to Pangolagrass they obtained a linear response to rates up to 288 pounds per acre; Racine (62), who obtained increased yields from Pangolagrass when he increased the rate of nitrogen added; Marchant (46), who obtained a satisfactory response from Pensacola Bahiagrass to nitrogen fertilization; Jeffers (34), who obtained good response from Pensacola Bahiagrass to high nitrogen levels; and Wallace et al. (83), who found increases in forage from Pangolagrass and Bahiagrass with each added increment of nitrogen.

Results obtained for Pangolagrass followed the generally expected trends more closely than did those for Bahiagrass. Although there were periodic differences in the hardiness of Pangolagrass at the two levels of nitrogen, there was a definite trend for more winter injury at the higher level of nitrogen. As in the case of Bahiagrass, there is reason to believe that no prolonged stages of hardening ever developed in Pangolagrass at either fertility level.

During the 1957-58 season, Pangolagrass appeared to develop cold hardiness after severe cold weather. The results indicate that, after periods of cold, differences in hardiness between the two fertility levels in Pangolagrass were less. Carroll and Welton (6), working with bluegrass, observed greater differences to exist between fertility levels after hardening than in the unhardened condition. The larger differences in susceptibility to cold between fertility levels in Pangolagrass existed in the unhardened condition rather than the hardened condition.

Conductivity levels during the 1958-59 season continually remained higher than 40 X $10^{-5}$ Mhos. In mid-October of 1957, Pangolagrass at the 100 pound level of nitrogen gave a reading this low. From a strict interpretation it would appear that in 1957 Pangolagrass was more hardy in mid-October at the 100 pound level of nitrogen than it ever was in the comparable period in 1958-59 at either fertility level.

Greenhouse recovery data indicate that at values of specific conductivity of 40 X $10^{-5}$ Mhos and above, Pangolagrass was not hardy enough to withstand the freezing treatment.

Precipitation was greater in 1957 in the six weeks preceding the study than in the same period preceding the 1958-59 study. Precipitation was greater during the period of study in 1958. Experiments reviewed by Levitt (41) would lead one to expect that under conditions of low environmental moisture hardening would be more pronounced. In the present study it is probable that the moisture present in the soil during both years was neither low enough nor high enough to bring about a stress which would have an effect on hardiness.

Throughout the 1958-59 season, the two fertility levels in Pangolagrass fluctuated in their degree of hardiness. As a general observation, Pangolagrass was hardier at the 100 pound level of nitrogen during the milder parts of the winter. Present data tend to be more in line with the work of Suneson and Peltier (84) who found that alternating temperatures were less effective for hardening of grains than constant low temperatures. This was assumed to be due to the continued growth under the conditions of alternating temperatures. Since Pangolagrass was especially prone to renew growth at the higher fertility level in warm periods following periods of cold, it suffered more loss of reserves in that the next freeze would kill the new top growth. This was also observed by Creel (15). Suneson and Peltier (84) found that high daily temperatures with high radiation were conducive to hardening of winter wheat. These conditions gave a maximum accumulation of organic reserves.

Salmon (60) and Lande (40) showed rather varied results in the amount of time required for loss of hardiness in winter hardened grains at greenhouse temperatures. The former found that five days were required for maximum loss while the latter found that 12 or 15 days were required. The present study shows that wide differences in the susceptibility of the grasses to cold could occur in a period of one week.

Temperature studies have shown that the rise and fall of hardiness in the grasses could not be correlated with either the average minimum temperature, the average mean temperature, or the lowest minimum temperature which occurred between observation dates, except in the case of Pangolagrass at the 400 pound level of nitrogen. In this latter case, a significant correlation was found between hardiness and both the average minimum and the lowest minimum temperature occurring between the dates of observation.

This would tend to show that Pangolagrass at the 400 pound level of nitrogen was very sensitive to temperature changes. In view of this and since periods of warm weather were often followed by periods of cold weather with frost, one may see that the loss of hardiness during the warm periods might leave the grass more susceptible to damage during the subsequent cold period. A parallel situation was observed in peach buds by Chaplin (12) who found that their killing point could rise as much as 11 degrees following a warm period.

Microclimate investigations provided information about the area immediately surrounding the plants. This information indicates that climatic data taken at the standard level of five feet does not represent the conditions existing in the microclimate. Soil surface temperatures under grass cover were found to be considerably higher than air temperatures at five feet while the nocturnal air temperatures at three inches were generally lower than the air temperature at five feet. Fluctuations in temperature were also wider at the three inch level. Following the pattern of nocturnal temperature inversion, the three-inch level was also observed to be usually several degrees cooler than the twelve-inch level. This would indicate that data taken in truck shelters in weather stations would also tend to give readings somewhat higher than the actual conditions occurring at night near the soil surface. Differences would probably be less likely to exist between levels on windy nights. This follows from the fact that wind was observed to reduce the temperature stratification.

## SUMMARY AND CONCLUSIONS

An experiment was conducted on the Agronomy farm at Gainesville, Florida from 1957 until 1959 to compare the influence of two rates of nitrogen fertilization on the cold hardiness of Pensacola Bahiagrass and Pangolagrass. This experiment followed preliminary studies carried out in the winter of 1956-57. The two rates of nitrogen were 100 and 400 pounds of nitrogen per acre per year respectively. Cold hardiness was studied in 1957-58 through the use of measurements of specific conductivity of solutions which contained plant material that had received a cold treatment. This treatment consisted of exposing plant material for a period of 24 hours in a deep freeze at 25° F. Following the cold treatment, deionized water was added to the samples and they were allowed 20 hours for diffusion of electrolytes. In 1958-59 the study utilized a modified process in which total conductivity was determined. The samples were ground in a Waring Blendor and the per cent of total conductivity exhibited by the samples following the freezing treatment was computed.

Greenhouse survival studies, following the freezing treatment, were carried on in 1957-58 in order to provide a means of checking the validity of the results of the conductivity experiments.

Statistical analyses were made of the data obtained in 1958-59.

Total nitrogen content was determined for the samples of grass which were tested for hardiness in 1958-59. Total nitrogen content was also determined for the forage samples obtained in the summer of 1958.

Climatic data were collected at the weather station located on the Agronomy farm. These included temperature data (taken at the five-foot level with maximum and minimum thermometers), precipitation data, and wind movement data.

Microclimatic data were obtained during periods of very cold weather. These temperatures were measured with a Brown recording potentiometer utilizing a sixteen-point system of copper-constantan thermocouples. Vertical levels of measurement included the soil surface, three inches in the air, twelve inches in the air, and five feet in the air.

Forage yield data were obtained in the summers of 1957 and 1958 and for the first cutting in 1959.

Comparisons of cold hardiness in 1957 and 1958 were made. In these comparisons, specific conductivity was used as the indicator. These comparisons showed that for Bahiagrass, hardiness was generally greater at the 400 pound level of nitrogen during both years. Pangolagrass generally appeared to exhibit greater cold hardiness at the 100 pound level during both years. The conductivity measurements show that apparently both grasses were more cold hardy during the 1957 season than during the 1958 season.

The 1958-59 season was divided into six periods of three observation dates each, and per cent of total conductivity was used as the indicator of hardiness. Periodic differences were observed to exist in both grasses at both fertility levels. Following periods of low temperatures, there was seen tendency for the grasses to exhibit degrees of cold hardiness at the two fertility levels in each grass which approached equality.

The cold hardiness studies indicated that Bahiagrass was more cold hardy at either fertility level than Pangolagrass at either fertility level.

More fluctuations between fertility levels as to degree of cold hardiness were noted in Pangolagrass. Wider spreads between fertility levels as to degree of cold hardiness were observed in Bahiagrass.

Greenhouse survival studies gave results which agreed with the results obtained from conductivity studies.

Essentially the same general trends were borne out during the two years of the study. Differences between the two years were probably largely attributable to the wide climatic differences experienced during the two winters.

Nitrogen content was not correlated with cold hardiness in either grass at either of the two fertility levels.

Yield data indicated that winter injury was more severe in Pangolagrass at the 400 pound level of nitrogen and that no differences existed between the fertility levels as to winter injury of Bahiagrass. During the more severe winter of 1957-58, Pangolagrass was more heavily damaged than in the 1958-59 winter while the opposite was true of Bahiagrass. Note is made of the fact that Bahiagrass was not damaged to any large extent in either winter.

Temperature studies showed that there was no correlation between cold hardiness and average minimum temperature, average mean temperature, or lowest minimum temperature when the temperatures used were those obtained between dates of experimental observation of cold hardiness, except in the case of Pangolagrass at the 400 pound level of nitrogen. In this latter case a correlation was found between cold hardiness and both the average minimum temperature and the lowest minimum temperature.

Other temperature studies, which involved microclimate investigations, determined that air temperatures were generally colder at night three inches above soil surface than at five feet above soil surface. The twelve-inch level was also found to be colder at night than the five-foot level. Soil surface under a grass cover was generally considerably warmer than the air at any of the levels observed. Fluctuations in range were greater at the three-inch level and at the twelve-inch level than at the other levels at which measurements were made. Wind was found to reduce temperature stratification.

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# BIOGRAPHICAL SKETCH

Leonard Hypret Dunavin, Jr. was born on December 17, 1930, in Algood, Tennessee. He attended school there and graduated from Algood High School on May 13, 1949.

In June, 1949, he entered the Tennessee Polytechnic Institute where he majored in agronomy. He was granted the Bachelor of Science degree, with distinction, from that institution on July 8, 1952.

He entered the University of Florida in September, 1952, to begin graduate work leading to the degree of Master of Science in Agriculture. This degree was granted on January 30, 1954. During this period he held the position of Graduate Assistant in the Department of Agronomy.

In May, 1954, he entered the Army of the United States and served for two years with the greater part of this period being spent in England.

Following his separation from the Army, he entered the University of Florida to begin work toward a doctor of philosophy degree with a major in agronomy and minors in soils and botany. During this period he held the positions of Agronomy Fellow and Research Assistant.

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This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 8, 1959

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